Is resistance to novobiocin a reliable test for confirmation of the identification of *Staphylococcus saprophyticus*?

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Summary. *Staphylococcus saprophyticus*, a coagulase-negative staphylococcus (CNS), causes acute urinary tract infection predominantly in young women (15–30 years). In the clinical microbiology laboratory identification and differentiation of *S. saprophyticus* from other CNS usually depends solely upon the demonstration of resistance to the antimicrobial agent novobiocin. Phenotypic characteristics of 36 novobiocin-resistant CNS isolated from the urine of patients with acute urinary tract infections were further analysed and the homogeneity of the isolates assessed. The organisms were speciated by the API STAPH identification system. Twenty-one isolates were *S. saprophyticus* ($p \geq 97\%$), and there was one strain each of *S. epidermidis*, *S. hominis* and *S. simulans* ($p \geq 97\%$). Of the remainder, three isolates were unidentifiable and a further nine had the characteristics associated with more than one species of CNS. Additional tests, including carbohydrate fermentation, antibiotic sensitivity and fluorogenic substrate utilisation, were performed on all isolates. Computer analysis of the results confirmed that testing for resistance to novobiocin selects a heterogeneous group of CNS composed of several different species.

Introduction

Coagulase-negative staphylococci (CNS), under certain conditions, may cause various infections that include intravascular-catheter-associated septicæmia, endocarditis and urinary tract infection (UTI).

In 1962, Torres Pereira isolated from urine a group of CNS that shared several phenotypic characteristics, including resistance to novobiocin at a concentration of 5 µg/ml. It was postulated that these CNS represented a newly recognised urinary tract pathogen. Maskell (1974) subsequently confirmed that a particular species of CNS, *Staphylococcus saprophyticus*, had a pathogenic role in community-acquired UTI, predominantly in young women.

The recognition of *S. saprophyticus* as a pathogen resulted in the requirement for CNS isolates from UTI to be identified to species level. Kloos and Schleifer (1975) had previously described a scheme for the identification and speciation of the genus *Staphylococcus*. The scheme involved the use of a variety of media and reagents and a requirement to incubate the organisms for prolonged periods. The

scheme was, therefore, unsuitable for routine clinical laboratories. Several abbreviated and rapid schemes for the identification of staphylococci have been developed subsequently (Brun *et al.*, 1978; Christensen *et al.*, 1983; Morgan, 1983; Bes *et al.*, 1985; Hussain *et al.*, 1986). These methods incorporated resistance to novobiocin at a concentration of 5 µg/ml for the presumptive identification of *S. saprophyticus*. However, it has now been recognised that other CNS are also resistant to novobiocin at this concentration, including *S. cohnii*, *S. sciuri* and *S. xylosus* (Hussain *et al.*, 1986).

In the present investigation, strains of presumptive *S. saprophyticus*, identified by novobiocin resistance, which had been isolated from patients with acute UTI, were examined further to evaluate the selectivity of this test. An extended range of identification tests was used not only to determine the homogeneity of this group of organisms but also to investigate the value of other potential methods for the identification of *S. saprophyticus*.

Materials and methods

Organisms

Thirty-six isolates of presumptive *S. saprophyticus* were obtained from the urine of patients with acute UTI. All

the organisms were present at a concentration of $\geq 10^5$ /ml of urine and were considered to be clinically significant. The CNS were identified by colonial appearance, Gram's stain, positive catalase reaction and negative coagulase and deoxyribonuclease (DNAase) reactions (Yu and Washington, 1985). A presumptive identification of *S. saprophyticus* was derived from resistance to novobiocin. This was performed by inoculation of DST agar (Oxoid) containing novobiocin (Sigma) 5 µg/ml. The presumptive *S. saprophyticus* isolates were further tested by the API STAPH biochemical strip (API Systems, Manuvix, France) to confirm the species identification.

Representative strains of other staphylococcal species described by Nachaie (1983) were kindly supplied by Dr M. Goodfellow, University of Newcastle upon Tyne. These strains had been isolated from human and veterinary sources worldwide. The isolates were included in the subsequent investigations for comparisons with the presumptive *S. saprophyticus* strains.

Carbohydrate fermentation tests

The staphylococci were tested for the ability to ferment carbohydrates (table I) by the replicator method described by Lennox and Ackerman (1984).

Antibiotic sensitivity tests

Antibiotic sensitivities of the staphylococcal isolates were determined by a replicator method. The isolates were multipoint inoculated on to DST Agar (Oxoid) plates containing one of the following antibiotics: chloramphenicol 10 µg/ml, erythromycin 2 µg/ml, gentamicin 4 µg/ml, neomycin 10 µg/ml, netilmicin 4 µg/ml, novobiocin 5 µg/ml, penicillin 0.1 µg/ml, tobramycin 4 µg/ml, tetracycline 2 µg/ml, or trimethoprim 1.25 µg/ml. Resistance was determined by growth of the isolate on the medium containing antibiotic.

The bacteria were also inoculated on to Phenolphthalein Phosphate Agar (Oxoid) to determine phosphatase activity.

Table I. Carbohydrates utilised in fermentation tests. The staphylococci were inoculated on to agar plates containing individual carbohydrates at a concentration of 20 g/L. Fermentation was determined by pH change as described by Lennox and Ackerman (1984)

L + arabinose	Me-β-D-glucopyranoside	ramnose
cellobiose	inositol	sorbitol
β-D-fructose	lactose	sucrose
galactose	maltose	trehalose
β-D-gentobiose	mannitol	turanose
glucose	mannose	xylitol
Me-α-D-glucopyranoside	D-raffinose	xylose

Enzyme profile

Various enzyme activities of the staphylococcal strains were determined. Non-fluorogenic derivatives of 7-amino-4-methylumbelliferone and 7-amino-4-methylcoumarin (tables II and III) were used to detect the presence of a series of enzymes. The derivatives consisted of various amino acids, organic and inorganic esters and glycosides bound to a methylumbelliferone or methylcoumarin marker. When specific enzymes were present which could degrade the covalent bond the fluorogenic moiety was released.

To determine the presence of specific enzymes the organisms were inoculated on to 5% horse blood agar (Difco) and incubated at 37°C for 18 h. A bacterial colony was then removed and emulsified with 20 µl of the fluorogenic substrate on Whatman's grade 1 filter paper which was incubated for 30 minutes at 37°C. The activity of an enzyme which had degraded carbohydrate was determined by the appearance of fluorescence when exposed to UV light.

Table II. The derivatives of 7-amino-4-methylumbelliferone used in the survey of phenotypic characteristics of staphylococci

<i>Organic esters:</i>	<i>Inorganic esters:</i>
oleate	bis-phosphate
palmitate	phosphate (acid)
stearate	phosphate (alkali)
	pyrophosphate
	sulphate
<i>Glycosides:</i>	
2-acetamido-2-deoxy- D-glucopyranoside	α-D-glucopyranoside
α-L-arabinofuranoside	β-D-glucopyranoside
β-L-arabinofuranoside	β-D-mannopyranoside
β-D-cellobiopyranoside	β-D-xyloside
β-D-fucopyranoside	N ₁ N ¹ -Diacetylchitobiose
α-D-galactopyranoside	2 ¹ -4 meu-K-D-N
β-D-galactopyranoside	acetylneuramic acid

Table III. The amino acid derivatives of 7-amino-4-methylcoumarin (AMC) used in the survey of phenotypic characteristics of isolates of staphylococci

L-ala-AMC	get-get-gly-phe-AMC
H-ala-AMC	H-met-AMC.AcOH
H-ala-ala-phe-AMC.HCl	H-orn-AMC
Z-arg-arg-AMC.2HCl	pyr-AMC
N-cbz-L-arg-AMC.HCl	H-ser-AMC.AcOH
H-asg-AMC.OH	H-thr-AMC
H-glu-AMC.OH	H-tyr-AMC
gly-AMC.HBr	L-val-AMC
Z-gly-gly-leu-AMC	Z-phe-arg-AMC
Z-gly-pro-AMC	boc-val-pro-arg-AMC
H-lys-AMC 2 acetate 1 AcOH	

Computer analysis

The results of the antibiotic sensitivities, carbohydrate fermentations and enzyme profile tests were analysed with the Clustan programme developed by Sneath (1957). The programme produced a dendrogram in which organisms with similar result profiles were grouped together.

The percentage of positive and negative results for each test carried out on the individual strains of staphylococci were determined. The species specificity of each test was then assessed.

Results

The 36 clinical isolates of presumptive *S. saprophyticus* identified by novobiocin resistance were further examined by the API STAPH biochemical strip. Data base analysis of the results confirmed that 21 isolates were *S. saprophyticus* ($p \geq 97\%$). Three of the isolates tested were unidentifiable. A further nine strains had characteristics attributable to more than one species of CNS and were given an indeterminate identification. The remaining three isolates were positively identified as *S. epidermidis*, *S. hominis* or *S. simulans* ($p \geq 97\%$).

Together with representative strains of other staphylococcal species the 36 clinical isolates were examined for the ability to ferment certain carbohydrates (tables IV and V), sensitivity to antibiotics (tables VI and VII) and for the capacity to utilise fluorogenic substrates (tables VIII, IX, X and XI). From these results, the organisms were subsequently grouped by Clustan analysis (figure) into 34 clusters (table XII) containing organisms with common phenotypic characteristics. Of the original 36 clinical isolates examined, 28 strains were allocated to six different clusters. Included within these six clusters were one strain of *S. cohnii* or *S. equorum*, and two strains of *S. xylosus*.

The remaining eight clinical isolates of presumptive *S. saprophyticus* were distributed throughout the other clusters. One isolate was grouped with three strains of *S. simulans* which were derived from the genito-urinary tract of patients. Another was related to the type strain of *S. chromogenes* and a third was allocated to a cluster containing a strain of *S. epidermidis* isolated from a UTI and two veterinary strains of *S. intermedius*. A further five isolates, including the type strain of *S. saprophyticus* (NCTC 7292), were unrelated to any of the other organisms tested.

The 21 isolates confirmed as *S. saprophyticus* by API STAPH were randomly distributed among the 34 clusters of staphylococci determined by Clustan analysis. Representatives of these 21 isolates were

not only grouped within the six clusters composed predominantly of strains of *S. saprophyticus* but were also associated with representative strains of other staphylococcal species (table XII).

The percentage positive results for each individual species in all of the tests was calculated. Representative results are shown in table XIII. These results show that the tests most discriminative for differentiating *S. saprophyticus* from the other species of staphylococci are: resistance to novobiocin 5 $\mu\text{g/ml}$; absence of phosphatase activity; aerobic fermentation of xylitol and maltose to produce acid; the utilisation of 2-acetamido-2-deoxy-D-glucopyranoside methylumbelliferone; α -L-arabinopyranoside-7-amino-4-methylumbelliferone; β -D-fucopyranoside-7-amino-4-methylumbelliferone; H-orn-7-amino-4-methylcoumarin; and pyr-7-amino-4-methylcoumarin. None of the tests performed in this study could alone adequately speciate *S. saprophyticus*.

Discussion

In the routine clinical laboratory, a urinary tract pathogen characterised as a CNS is presumptively identified as *S. saprophyticus* on the basis of a single test—resistance to novobiocin. Other CNS, e.g., *S. cohnii*, *S. sciuri* and *S. xylosus*, are also resistant to novobiocin at a concentration of 5 $\mu\text{g/ml}$ (Yu and Washington, 1985). These organisms are not commonly associated with UTI, although *S. xylosus* has been implicated in urinary infections in men (Tselenis-Kotsowilis *et al.*, 1982). Also, antibiotic resistance in staphylococci is often encoded on plasmids and, as such, is available for inter-species transfer. Care should be taken, therefore, to ensure accurate speciation.

The API STAPH identification system, which does not incorporate a test for resistance to novobiocin, determined that three of the 36 clinical isolates studied were strains of *S. epidermidis*, *S. hominis* and *S. simulans* ($p \geq 97\%$). In the extended survey, which incorporated carbohydrate fermentation, antibiotic sensitivity (including resistance to novobiocin), and fluorogenic substrate tests, individual presumptive *S. saprophyticus* isolates possessed similar reaction profiles to the species *S. cohnii*, *S. chromogenes*, *S. intermedius*, *S. xylosus* and *S. simulans*. Conversely, other species of staphylococci shared phenotypic similarities with *S. saprophyticus*. This finding illustrates the heterogeneity of novobiocin-resistant CNS associated with UTI.

Nine clinical isolates tested by API STAPH possessed profile numbers which were equally

Table IV. The fermentation of selected carbohydrates by clinical isolates of *S. saprophyticus*

Strain no.	Fermentation (acid production, +) of																				
	L + arabinose	cellobiose	β -D-fructose	galactose	β -gentobiose	Me- α -D-glucopyranoside	Me- β -D-glucopyranoside	glucose	inositol	lactose	maltose	mannitol	D + mannose	D + raffinose	rhamnose	sorbitol	sucrose	trehalose	turanose	xylitol	xylose
L002	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L003	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L004	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	+	-
L005	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L006	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L007	-	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	+	+	-	-	-
L010	-	-	+	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	-	+	-
L011	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	+	-
L014	-	-	+	-	-	-	+	+	-	+	-	+	-	-	-	-	+	+	-	-	-
L016	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L017	-	-	+	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	-	+	+
L018	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	-	+	+	+	+	+
L019	-	-	+	-	-	-	+	+	-	-	+	-	-	-	-	-	+	-	-	-	-
L020	-	-	+	-	+	-	+	+	-	+	+	+	-	-	-	-	+	+	-	+	-
L021	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-
L022	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-
L024	-	-	+	+	-	-	+	+	-	+	-	-	+	-	-	-	+	+	-	-	-
L025	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L026	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L027	-	-	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	-	-	+
L029	-	-	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	-	-	-
L030	-	-	+	-	+	-	+	+	-	+	+	+	-	-	-	-	+	+	-	-	+
L032	-	-	+	-	+	-	+	+	-	-	+	+	-	-	-	-	+	+	-	+	-
L034	+	-	+	-	-	-	-	+	-	+	+	+	-	-	+	-	+	+	-	+	-
L035	-	-	+	-	-	-	-	+	-	-	+	+	-	-	-	+	+	+	-	+	-
L036	-	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	+	+	-	+	-
L037	-	-	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	-	-	-
L039	-	-	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	-	+	-
L040	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L041	-	-	+	-	+	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L042	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L043	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
L044	-	-	+	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	+	+	+
L056	-	-	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	-	-	-
L059	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
L031	-	-	+	-	+	-	+	+	-	+	+	+	-	-	-	-	+	+	-	+	-

representative of more than one species of staphylococcus. In this situation the API STAPH identification system recommends testing for resistance to novobiocin, thereby enabling differentiation of *S. saprophyticus* from other CNS. There is, therefore, total reliance on a single characteristic for speciation since confirmation of the identity of *S.*

saprophyticus is based upon the same test as the initial presumptive identification.

Conventional identification tests such as resistance to novobiocin and phosphatase activity were found to be particularly useful in differentiating *S. saprophyticus* from *S. epidermidis*. Additional tests which may prove useful in the differentiation of *S.*

Table V. The fermentation of selected carbohydrates by reference strains of staphylococci

Strain no.		Fermentation (acid production, +) of																	
		L + arabinose	cellobiose	D + fructose	galactose	β -gentobiose	Me- α -D-glucopyranoside	Me- β -D-glucopyranoside	glucose	inositol	lactose	maltose	mannitol	D + mannose	D + raffinose	rhamnose	sorbitol	sucrose	trehalose
<i>S. aureus</i>	R001	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	R002	-	-	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	+
	R006	+	+	+	+	-	-	-	+	+	-	-	+	-	-	+	-	+	+
<i>S. capitis</i>	R026	-	+	+	-	-	-	+	+	-	-	-	+	-	-	+	-	+	+
<i>S. caprae</i>	R029	-	-	+	-	-	-	+	+	+	+	+	+	-	-	+	-	+	+
	R290	-	-	+	-	+	-	-	+	-	-	+	+	+	+	-	+	+	+
<i>S. caseolyticus</i>	R030	-	-	+	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+
<i>S. chromogenes</i>	R031	-	-	+	-	-	-	-	+	-	+	+	+	-	-	-	+	+	+
	R036	-	-	+	-	+	-	-	+	-	+	-	-	-	-	-	+	+	-
<i>S. cohnii</i>	R053	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	-	+	+
<i>S. epidermidis</i>	R056	-	-	+	+	-	-	-	+	-	-	+	+	-	-	-	-	+	+
	R061	-	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+	+	+
	R063	-	-	+	-	-	-	+	+	-	+	+	+	+	-	-	-	+	-
	R160	-	-	+	-	-	-	+	+	-	+	+	+	+	-	-	-	+	-
	R243	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
	R246	-	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	+	+
<i>S. equorum</i>	R170	-	-	+	-	-	-	-	+	-	+	+	+	+	-	-	-	+	+
<i>S. gallinarum</i>	R171	-	-	+	-	-	-	-	+	-	+	+	+	+	-	-	-	+	+
<i>S. haemolyticus</i>	R071	-	-	+	+	+	-	-	+	-	+	+	+	-	-	+	-	+	-
	R073	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+
<i>S. hominis</i>	R075	-	+	+	-	-	+	+	+	-	+	-	+	+	-	-	-	+	+
	R076	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+
	R077	-	-	+	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+
<i>S. hyicus</i>	R089	-	-	+	-	-	-	+	+	-	+	-	-	+	-	-	-	+	+
<i>S. intermedius</i>	R097	-	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	+	+
	R101	-	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	+	+
<i>S. simulans</i>	R117	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	-	+	+
	R118	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	+	+
	R120	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
	R129	-	-	+	-	-	-	+	+	-	-	+	+	+	-	-	-	+	+
<i>S. warneri</i>	R133	-	-	+	-	-	-	-	+	-	+	-	+	+	-	-	-	+	+
	R134	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+
<i>S. xylosus</i>	R137	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+
	R147	-	-	+	-	+	-	-	+	-	-	-	+	+	-	-	-	+	-
	R181	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+
<i>S. kloosii</i>	R185	+	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	+	+
	R305	-	-	+	-	-	-	-	+	-	+	-	+	-	-	-	-	+	+

saprophyticus from other CNS have been determined as a result of the present work. These include the ability to cleave β -D-fucopyranoside from fluorogenic 7-amino-4-methylumbelliferone, a property attributable to more strains of *S. saprophyticus* than of other staphylococcal species studied. Unlike the Kloos and Schleifer system, these tests are rapid and easy, require only standard laboratory

equipment, and so are useful in the routine clinical laboratory. The fluorogenic substrate tests are particularly rapid and yield results within 1 h of testing.

These findings suggest that tests for resistance to novobiocin and absence of phosphatase activity should both be performed in the routine clinical microbiology laboratory to differentiate *S. sapro-*

Table VI. The antibiotic resistance pattern, DNAase and phosphatase activities of *S. saprophyticus*

Strain no.		Results obtained in tests with												
		phosphatase	DNAase	novobiocin	penicillin	cloxacillin	tetracycline	erythromycin	trimethoprim	tobramycin	gentamicin	netilmicin	neomycin	chloramphenicol
<i>S. saprophyticus</i>	LO02	-	-	R	R	S	S	S	S	R	R	S	R	S
	LO03	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO04	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO05	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO06	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO07	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO10	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO11	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO14	-	-	R	R	S	S	S	S	S	S	S	S	S
	LO16	-	-	R	R	S	R	S	S	S	S	S	S	S
	LO17	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO18	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO19	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO20	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO21	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO22	-	-	R	R	S	S	S	S	S	S	S	S	S
	LO24	-	-	R	R	S	S	S	S	S	S	S	S	S
	LO25	-	-	R	R	R	R	S	S	S	S	S	S	S
	LO26	-	-	R	R	R	R	R	S	S	S	S	R	R
	LO27	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO29	-	-	R	R	S	S	S	S	S	S	S	S	S
	LO30	-	-	R	R	S	S	S	S	S	S	S	S	S
	LO32	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO34	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO35	-	-	R	R	R	R	S	S	S	S	S	R	R
	LO36	-	-	R	R	S	S	S	S	S	S	S	S	S
	LO37	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO39	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO40	-	-	R	R	S	S	S	S	S	S	S	S	S
	LO41	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO42	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO43	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO44	-	-	R	R	R	S	S	S	S	S	S	S	S
	LO56	-	-	R	S	S	S	S	S	S	S	S	S	S
	LO59	-	-	R	S	S	S	S	S	S	S	S	S	S
	LO31	-	-	R	S	S	S	S	S	S	S	S	S	S

+, Positive result DNAase, phosphatase activity; R, resistance to the antibiotic; S, sensitivity to the antibiotic.

phyticus from *S. epidermidis*. In studies requiring accurate speciation of *S. saprophyticus*, for example the role of *S. saprophyticus* in the pathogenesis of UTI in young women, further tests should be performed as suggested in this present study.

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Table VII. The antibiotic resistance pattern, DNAase and phosphatase activities of reference strains of staphylococci

Strain no.		Results obtained in tests with												
		phosphatase	DNAase	novobiocin	penicillin	cloxacillin	tetracycline	erythromycin	trimethoprim	tobramycin	gentamicin	netilmicin	neomycin	chloramphenicol
<i>S. aureus</i>	R001	+	+	S	R	S	S	S	S	S	S	S	S	S
	R002	+	+	S	R	S	S	S	S	S	S	S	S	S
	R006	+	+	S	R	S	R	S	S	S	S	S	S	S
<i>S. capitis</i>	R026	+	+	S	S	S	S	S	S	S	S	S	S	S
<i>S. caprae</i>	R029	+	+	S	S	S	S	S	S	S	S	S	S	S
	R290	+	+	S	S	S	S	S	S	S	S	S	S	S
<i>S. caseolyticus</i>	R030	+	+	S	S	S	S	S	S	S	S	S	S	S
<i>S. chromogenes</i>	R031	+	+	S	S	S	S	S	S	S	S	S	S	S
	R036	+	+	S	S	S	S	S	S	S	S	S	S	S
<i>S. cohnii</i>	R053	+	+	R	R	R	S	S	S	S	S	S	S	S
<i>S. epidermidis</i>	R056	+	+	S	R	S	R	S	S	S	S	S	S	S
	R061	+	+	S	R	S	R	S	R	R	S	S	S	S
	R063	+	+	R	R	R	S	S	R	R	S	S	S	S
	R160	+	+	S	R	S	R	S	S	S	S	S	S	S
	R243	+	+	S	R	S	R	S	S	S	S	S	S	S
	R246	+	+	S	S	S	R	S	S	S	S	S	S	S
	R170	+	+	R	R	R	R	S	S	S	S	S	S	S
<i>S. equorum</i>	R171	+	+	R	R	R	R	S	S	S	S	S	S	R
<i>S. gallinarum</i>	R071	+	+	R	R	S	R	S	R	R	R	R	S	R
<i>S. haemolyticus</i>	R073	+	+	S	R	S	R	S	R	R	S	S	R	S
	R075	+	+	S	S	R	S	S	S	S	S	S	S	S
<i>S. hominis</i>	R076	+	+	R	S	S	S	S	S	S	S	S	R	S
	R077	+	+	R	S	R	S	S	S	S	S	S	R	S
<i>S. hyicus</i>	R089	+	+	S	S	R	R	S	S	S	S	S	S	R
<i>S. intermedius</i>	R097	+	+	S	S	R	S	S	S	S	S	S	S	S
	R101	+	+	S	S	S	S	S	S	S	S	S	S	S
<i>S. simulans</i>	R117	+	+	R	R	R	S	S	R	S	S	S	S	R
	R118	+	+	R	S	S	S	S	S	S	S	S	S	S
	R120	+	+	S	S	R	S	S	S	S	S	S	S	S
	R128	+	+	R	S	S	S	R	S	S	S	S	S	S
<i>S. warneri</i>	R133	+	+	S	S	S	S	S	S	S	S	S	S	S
	R134	+	+	S	R	S	S	S	S	S	S	S	S	S
<i>S. xylosus</i>	R137	+	+	R	R	S	S	S	S	S	S	S	S	S
	R147	+	+	R	R	R	R	S	S	S	S	S	S	S
<i>S. kloosii</i>	R181	+	+	R	R	S	S	S	S	S	S	S	S	S
	R185	+	+	R	R	S	S	S	S	S	S	S	S	S
	R305	+	+	R	R	S	S	S	S	S	S	S	S	S

+, Positive DNAase, phosphatase activity; R, resistance to the antibiotic; S, sensitivity to the antibiotic.

Table VIII. The utilisation of derivatives of 7-amino-4-methylumbelliferone by 36 strains of *S. saprophyticus*

Strain no.	Derivatives metabolised																				
	palmitate	stearate	oleate	2-acetamido-2-deoxy-D-glucopyranoside	α -L-arabinofuranoside	β -L-arabinofuranoside	β -D-cellobiopyranoside	β -D-fucopyranoside	α -D-galactopyranoside	β -D-galactopyranoside	α -D-glucopyranoside	β -D-glucopyranoside	β -D-mannopyranoside	β -D-xyloside	N ₁ N ¹ -diacetyl chitobioside	2'-4 meu-K-D-N-acetylneuramic acid	bis-phosphate (acid)	phosphate (acid)	phosphate (alkali)	pyrophosphate	sulphate
L002	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
L003	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
L004	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
L005	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
L006	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L007	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L010	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+
L011	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
L014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
L016	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
L017	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
L018	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
L019	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+
L020	+	+	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+
L021	+	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	+	+
L022	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+
L024	+	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
L025	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+
L026	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
L027	+	+	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+
L029	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+
L030	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+
L032	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	+
L034	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+
L035	+	+	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+
L036	+	+	-	+	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	+	+
L037	+	+	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	+	+
L039	+	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	+	+	+	+
L040	+	+	-	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+	+
L041	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+
L042	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+
L043	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	+	-	+	+
L044	+	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	+	+
L056	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
L059	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	+	+	+
L031	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-	-

Table IX. The utilisation of 7-amino-4-methylumbelliferone derivatives by reference strains of staphylococci

Strain no.		palmitate	stearate	oleate	2-acetamido-2-deoxy-D-glucopyranoside	α -L-arabinofuranoside	β -L-arabinofuranoside	β -D-cellobiopyranoside	β -D-fucopyranoside	α -D-galactopyranoside	β -D-galactopyranoside	α -D-glucopyranoside	β -D-glucopyranoside	β -D-mannopyranoside	β -D-xyloside	N ₁ N ₁ '-diacetyl chitobioside	2'-4' meu-K-D-N-acetylneuramic acid	bis-phosphate (acid)	phosphate (acid)	phosphate (alkali)	pyrophosphate	sulphate
<i>S. aureus</i>	R001	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
	R002	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R006	+	+	+	+	-	+	-	-	-	-	-	-	+	-	-	+	-	+	-	-	+
<i>S. capitis</i>	R026	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
<i>S. caprae</i>	R029	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
	R290	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	R030	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>S. caseolyticus</i>	R031	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	R036	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R053	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>S. epidermidis</i>	R056	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
	R061	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-
	R063	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
	R160	+	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+
	R243	+	+	-	+	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	+	+
	R246	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+
	R170	+	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+
<i>S. equorum</i>	R171	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
<i>S. gallinarum</i>	R071	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
	R073	+	+	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+
	R075	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+
<i>S. hominis</i>	R076	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	R077	+	+	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+
	R089	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+
<i>S. hyicus</i>	R097	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	R101	+	+	-	+	-	+	-	+	+	-	+	-	-	+	-	-	-	-	-	+	+
	R117	+	+	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-	+
<i>S. simulans</i>	R118	+	-	-	-	-	+	-	-	-	-	+	+	-	+	-	-	+	-	+	-	+
	R120	+	+	-	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+
	R129	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>S. warneri</i>	R133	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+
	R134	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+	+	+
	R137	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+
<i>S. xylosus</i>	R147	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	R181	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	+	+
	R185	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	-
<i>S. kloosii</i>	R305	+	+	-	+	-	+	-	+	+	-	+	+	-	+	-	+	+	-	+	+	+

Table X. The utilisation of 7-amino-4-methylcoumarin (AMC)-derivatives by *S. saprophyticus*

[illegible]

Table XI. The utilisation of 7-amino-4-methylcoumarin (AMC) derivatives by reference strains of staphylococci

Strain no.		Derivatives metabolised																					
		L-ala-AMC	H-ala-AMC	H-ala-ala-phe-AMC.HCl	Z-arg-arg-AMC.2HCl	N-cbz-L-arg-AMC.HCl	H-asp-AMC.OH	H-glu-AMC.OH	gly-AMC.HBr	Z-gly-gly-leu-AMC	Z-gly-pro-AMC	H-lys-AMC 2acetate 1 AcOH	get-get-gly-phe-AMC	H-met-AMC.AcOH	H-orn-AMC.H ₂ O	pyr-AMC	H-ser-AMC.AcOH	H-thr-AMC	H-tyr-AMC	L-val-AMC	Z-phe-arg-AMC.HCl	boc-val-pro-arg-AMC	
<i>S. aureus</i>	R001	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	
	R002	+	-	+	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	+	
	R006	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>S. capitis</i>	R026	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	-	-	-	-	
<i>S. caprae</i>	R029	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	
	R290	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>S. caseolyticus</i>	R030	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	
<i>S. chromogenes</i>	R031	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	
	R036	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>S. cohnii</i>	R053	+	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	
<i>S. epidermidis</i>	R056	+	-	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	
	R061	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	
	R063	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
	R160	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	
	R243	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
	R246	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	
<i>S. equorum</i>	R170	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>S. gallinarum</i>	R171	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
<i>S. haemolyticus</i>	R071	+	-	+	-	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	-	
	R073	+	-	+	-	-	+	-	+	-	+	-	+	+	-	-	+	-	+	-	-	-	
	R075	-	-	+	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	
<i>S. hominis</i>	R076	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	
	R077	+	-	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	
	R089	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	
<i>S. hyicus</i>	R097	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
	R107	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
	R117	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
<i>S. simulans</i>	R118	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
	R120	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	
	R129	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	
	R133	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	
<i>S. warneri</i>	R134	+	-	-	-	-	-	-	+	-	+	-	+	-	-	+	+	-	-	-	-	-	
	R137	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>S. xylosus</i>	R147	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	R181	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
<i>S. kloosii</i>	R185	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	R305	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	

Figure. Dendrogram produced by Clustan analysis illustrating the percentage similarity of the test strains.

Table XII. The 34 distinct clusters of related organisms obtained by Clustan analysis based upon staphylococcal phenotypic characteristics. When more than one strain of a particular species was investigated the number is shown in brackets. The cluster numbers represent the similarity of species within each cluster, consecutive groups being more closely related.

1. <i>S. aureus</i>	11. <i>S. saprophyticus</i> (7/4†)	23. <i>S. xylosus</i>
<i>S. epidermidis</i>	12. <i>S. saprophyticus</i> (3/2†)	24. <i>S. saprophyticus</i> (1/1†)
<i>S. chromogenes</i>	<i>S. xylosus</i>	25. <i>S. saprophyticus</i> (2/0†)
2. <i>S. capitis</i>	13. <i>S. gallinarum</i>	26. <i>S. saprophyticus</i>
<i>S. warneri</i>	14. <i>S. hominis</i>	27. <i>S. saprophyticus</i> *
3. <i>S. caprae</i>	15. <i>S. simulans</i>	28. <i>S. epidermidis</i>
<i>S. caseolyticus</i>	<i>S. saprophyticus</i> (1/1†)	29. <i>S. haemolyticus</i>
4. <i>S. hominis</i>	16. <i>S. epidermidis</i>	30. <i>S. haemolyticus</i>
5. <i>S. saprophyticus</i> (2/1†)	17. <i>S. kloosi</i>	31. <i>S. aureus</i>
6. <i>S. chromogenes</i>	18. <i>S. saprophyticus</i> (3/10†)	32. <i>S. lentus</i>
7. <i>S. kloosi</i>	<i>S. xylosus</i>	33. <i>S. lentus</i>
<i>S. saprophyticus</i> (1/1†)	19. <i>S. saprophyticus</i> (2/2†)	34. <i>S. hyicus</i>
8. <i>S. epidermidis</i>	20. <i>S. hominis</i>	
<i>S. intermedius</i> (2)	21. <i>S. simulans</i>	
<i>S. saprophyticus</i> (1/1†)	22. <i>S. aureus</i>	
9. <i>S. saprophyticus</i> (3/1†)		
10. <i>S. saprophyticus</i> (10/8)†		
<i>S. cohnii</i>		
<i>S. equorum</i>		

* Type strain of *S. saprophyticus* (NCTC 7292).

† Number of *S. saprophyticus* strains with identification confirmed by API STAPH.

Table XIII. Percentage of strains of staphylococcal species able to ferment carbohydrates, utilise fluorogenic substrates and resistant to novobiocin 5 µg/ml. Results for the 36 presumptive *S. saprophyticus* strains identified in the clinical laboratory and the 21 confirmed isolates are shown as original and confirmed respectively

Species	Number of strains tested	Percentage of strains that gave positive results in tests								
		M10	M14	M16	S10	S22	A18	A19	NOV	PHOS
<i>S. saprophyticus</i> (original)	36	58.3	58.3	18	33.3	58.3	18	58.3	100	
<i>S. saprophyticus</i> (confirmed)	21	60.8	69.5	56.5	43.3	91	17.3	43.4	100	
<i>S. aureus</i>	3	33.3	33.3					100		100
<i>S. capitis</i>	1									100
<i>S. caprae</i>	2		50		50	100	100	100		100
<i>S. caseolyticus</i>	1				100	100	100	100		
<i>S. chromogenes</i>	2	50	50			50	50	50		
<i>S. cohnii</i>	1	100				100				
<i>S. epidermidis</i>	6		33.3	16	16	66.6	16	50	33.3	100
<i>S. equorum</i>	1	100	100			100			100	100
<i>S. gallinarum</i>	1					100		100	100	
<i>S. haemolyticus</i>	2	50	50		50	100		100	50	
<i>S. hominis</i>	3		33.3		33.3	33.3	100	33.3	100	
<i>S. hyicus</i>	1				100			100		
<i>S. intermedius</i>	2	100	100	50		100		100		100
<i>S. kloosii</i>	2		50						100	
<i>S. simulans</i>	4	50	25	25	25			100	75	50
<i>S. warneri</i>	2			50						
<i>S. xylosus</i>	3		33.3	100				66.6	100	100

M10—utilisation of 2 acetamido-2-deoxy-D-glucopyranoside.

M14—utilisation of α-L-arabinopyranoside-7-amino-4-methylumbelliferone.

M16—utilisation of β-D-fucopyranoside-7-amino-4-methylumbelliferone.

S10—fermentation of xylitol.

S22—fermentation of maltose.

A18—utilisation of H-orn-7-amino-4-methylcoumarin.

A19—utilisation of pyr-7-amino-4-methylcoumarin.

NOV—resistance to novobiocin 5 µg/ml.

PHOS—phosphatase activity.

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