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Some properties of coagulase-negative staphylococci isolated from cases of ovine mastitis

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SUMMARY

Of 41 coagulase-negative staphylococcal isolates from cases of ovine mastitis, 80% were speciated by the 'API-Staph SYSTEM' and 90% by a combination of biochemical tests. *Staphylococcus simulans* and *Staph. xylosus* were the two most prevalent species.

INTRODUCTION

The taxonomy of coagulase-negative staphylococci (CNS) has been revised in recent years with the definition of new species [1]. Traditionally, CNS have been considered as non-pathogenic or of low pathogenicity for the mammary gland [2], although recently Fthenakis [3] showed that CNS caused clinical and subclinical mastitis in sheep.

In this paper we report on some laboratory characteristics and sensitivity to antimicrobial agents of CNS isolated from ovine mastitis.

MATERIALS AND METHODS

Staphylococcal isolates

A collection of 41 isolates from the mammary secretion of ewes with acute clinical mastitis (isolates 1–19), subclinical mastitis (20–36) or chronic clinical mastitis detected after weaning (37–41) were obtained during a survey of ovine mastitis in England and Wales [4].

Identification procedure

Isolates were considered to be CNS if they were Gram-positive cocci, catalase-positive and coagulase-negative by tube and slide tests, with rabbit and sheep plasma (Sigma Chemical Company Ltd). Lysostaphin sensitivity ('Lysostaphin Test', Roche Products Ltd) and anaerobic fermentation of glucose were used to differentiate CNS from micrococci.

Each isolate was subcultured on Columbia blood agar, containing 5%

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defibrinated sheep blood, and incubated aerobically and anaerobically at 37 °C. Growth of bacteria after 18 h and the appearance of the colonies after aerobic incubation were recorded. The production of DNAse and phosphatase and the cytochrome C oxidase activity were assessed [5, 6].

The isolates were speciated using the 'API-Staph SYSTEM' (API Laboratory Products Ltd) and a combination of biochemical tests [5]. Species identification (according to the 'API-Staph SYSTEM') was accepted, if the degree of probability was greater than 75%.

Antimicrobial agents sensitivity tests

All isolates were tested by a disk method [5]. Disks containing chloramphenicol (10 μg), erythromycin (10 μg), fucidin (5 μg), gentamicin (10 μg), lincomycin (2 μg), methicillin (10 μg), neomycin (10 μg), novobiocin (5 μg), penicillin-G (1 i.u.), streptomycin (10 μg), tetracycline (10 μg) and trimethoprim (1·25 μg) were used.

RESULTS

Speciation

All isolates grew aerobically and anaerobically after 18 h incubation. Colonies were generally about 2 mm in diameter after aerobic incubation, but large (> 2 mm), small (< 2 mm) and pinpoint (< 1 mm) colonies were also seen. Colonies of all isolates were smooth, entire and raised, except those of five isolates (Table 1; 8, 13, 19, 25, 40), which were flat. Most colonies were white, but buff, golden and grey pigmentation was encountered; α-haemolysis was recorded for six isolates (Table 1; 1, 2, 17, 19, 20, 23).

Twenty-one different profiles were recorded using the 'API-Staph SYSTEM' and 33 (80%) isolates were speciated. Ten were *Staph. simulans* (profiles 6432153, 6436153, 6532053, 6532153), 9 *Staph. xylosus* (profiles 6736452, 6736453, 6734453, 6776452), 5 *Staph. chromogenes* (profiles 6516013, 6716053), 4 *Staph. epidermidis* (profiles 6706013), 3 *Staph. auricularis* (profile 6000001), 1 was *Staph. haemolyticus* (profile 6632111) and 1 *Staph. sciuri* (profile 6732110). The following profiles were also recorded: 6132410, 6232011, 6634113, 6716050, 6734103, 6736103, 6736113.

Thirty-seven (90%) isolates were speciated using the combination of biochemical tests (Table 1). Nine were *Staph. simulans*, 9 *Staph. xylosus*, 8 *Staph. hyicus*, 4 *Staph. epidermidis*, 4 *Staph. haemolyticus*, 1 *Staph. capitis*, 1 *Staph. saprophyticus* and 1 *Staph. werneri*.

The correspondence of speciation by the two methods used is presented in Table 2. All *Staph. xylosus* and *Staph. epidermidis* isolates were similarly identified by both methods. All, but one, isolates identified as *Staph. simulans* using the 'API-Staph SYSTEM' were similarly speciated using the combination of biochemical tests. All isolates speciated as *Staph. chromogenes* using the 'API-Staph SYSTEM' were identified as *Staph. hyicus* using the biochemical tests, as were another four isolates not speciated by the 'API-Staph SYSTEM'.

Of the 19 isolates from acute clinical mastitis, 7 were *Staph. xylosus*, 4 *Staph. simulans* and 3 *Staph. chromogenes* (or *Staph. hyicus*). Of the 17 isolates from subclinical mastitis, 4 were *Staph. epidermidis*, 2 *Staph. xylosus* and 2 *Staph. simulans*.

Table 1. *The results of speciation of 41 CNS isolates using biochemical tests*

Isolates	O/F	Ar/Xy.	Lact.	Malt.	Mann.	Sucr.	Treh.	Acet.	Nit.	DNAse	Phos.	Species
1, 2, 37, 39	F	−	+	−	+	+	+	−	+	+	−	*S. simulans*
12	F	−	+	−	+	+	+	−	+	−	−	*S. simulans*
17, 41	F	−	+	−	+	+	+	−	+	+	+	*S. simulans*
22	F	−	+	−	+/−	+	+	−	+	−	+	*S. simulans*
23	F	−	+	−	+	+	+	−	+	−	+	*S. simulans*
4	−/−	+	+	+/−	+	+	+	−	−	−	−	*S. xylosus*
6	F	+	+	+	+	+	+	−	+	−	+	*S. xylosus*
11	F	+/−	+	+/−	+	+	+	−	+	−	+	*S. xylosus*
14	O	+	+	+	+	+	+	−	−	+	+	*S. xylosus*
15	O	+	+	+	+	+	+	−	−	−	+	*S. xylosus*
16	O	+	+	+	+	+/−	+	−	−	+	+	*S. xylosus*
18	O	+	+	+	+	+	+	−	+	−	−	*S. xylosus*
31	O	+	+	+	+	+	+	−	+	−	+	*S. xylosus*
32	O	+	+	+	+	+	+	−	+	+	+	*S. xylosus*
8, 10, 13	F	−	+	−	−	+	+/−	−	+	+	+	*S. hyicus*
9, 25	F	−	+	−	−	+	+	−	+	+	+	*S. hyicus*
19	F	−	+	−	−	+	−	−	+	+	+	*S. hyicus*
38	F	−	+	−	−	+	+	−	+	+	−	*S. hyicus*
40	F	−	+	−	−	+	+	−	+	−	+	*S. hyicus*
33, 34, 35, 36	F	−	+	+	−	+	−	+	+	+	−	*S. epidermidis*
21, 28	F	−	+	+	+	+	+	+	+	−	−	*S. heamolyticus*
29	F	−	+	+	+	+	+	+	+	+	+	*S. haemolyticus*
30	F	−	+	+	+	+	+	+	−	+	−	*S. haemolyticus*
20	F	−	−	−	+	−	+	+	+	−	−	*S. capitis*
7	F	−	−	−	+	−	+	−	+	−	−	*S. saprophyticus*
5	F	−	−	+	+	−	+	+	+	−	−	*S. warneri*
3	−/−	−	−	−	−	−	−	−	+	−	−	*Staph.* sp.
24	F	−	−	−	−	−	−	−	+	+	−	*Staph.* sp.
26, 27	O	−	−	−	−	−	−	−	−	−	−	*Staph.* sp.

O/F: oxidative or fermentative organism. F, fermentative; O, oxidative; +, positive; −, negative.
Ar/Xy., arabinose/xylose fermentation; Lact., lactose fermentation; Malt., maltose fermentation; Mann., mannose fermentation; Sucr., sucrose fermentation; Treh., trehalose fermentation; Acet., acetoin production; Nit., nitrate reduction; Dnase, DNAse production; Phos., phosphatase production.

Table 2. *Correspondence of speciation using the 'API-Staph SYSTEM' and the combination of biochemical tests*

	'API-Staph SYSTEM'	Biochemical tests
Isolates from acute clinical mastitis		
1, 2, 12, 17	*S. simulans*	*S. simulans*
3	*Staph.* sp.	*Staph.* sp.
4, 6, 11, 14, 15, 16, 18	*S. xylosus*	*S. xylosus*
5	*Staph.* sp.	*S. warneri*
7	*Staph.* sp.	*S. saprophyticus*
8, 13, 19	*S. chromogenes*	*S. hyicus*
9, 10	*Staph.* sp.	*S. hyicus*
Isolates from subclinical mastitis		
20	*Staph.* sp.	*S. capitis*
21	*S. sciuri*	*S. haemolyticus*
22, 23	*S. simulans*	*S. simulans*
24, 26, 27	*S. auricularis*	*Staph.* sp.
25	*S. chromogenes*	*S. hyicus*
28	*S. haemolyticus*	*S. haemolyticus*
29, 30	*Staph.* sp.	*S. haemolyticus*
31, 32	*S. xylosus*	*S. xylosus*
33, 34, 35, 36	*S. epidermidis*	*S. epidermidis*
Isolates from chronic clinical mastitis		
37, 39, 41	*S. simulans*	*S. simulans*
38, 40	*S. simulans*	*S. hyicus*

Sensitivity to antimicrobial agents

All 9 *Staph. xylosus* isolates were resistant to lincomycin and 8 to fucidin, novobiocin and penicillin-G (*Staph. xylosus* isolates are naturally resistant to novobiocin and usually resistant to lincomycin). Of the *Staph. simulans* isolates, 2 were resistant to lincomycin, 1 to fucidin and 1 to trimethoprim. None of the *Staph. hyicus* (or *Staph. chromogenes*) isolates was resistant to any antibiotic tested. All 4 *Staph. epidermidis* isolates were resistant to penicillin and tetracycline, 3 to methicillin and 2 to streptomycin.

DISCUSSION

Most investigations of CNS deal with isolations from people and consequently most of the staphylococcal species presently recognized are primarily of medical interest, although some have been considered additionally as animal pathogens.

The most prevalent CNS species isolated from the milk of healthy cows include *Staph. xylosus*, *Staph. epidermidis*, *Staph. sciuri* [7, 8] and those from cows with mastitis *Staph. epidermidis*, *Staph. hyicus*, *Staph. simulans* [9–13]. The most prevalent CNS species isolated from goats with subclinical mastitis was *Staph. epidermidis* [14]. Guitierrez and others [15] identified 5 (of the 8 examined) CNS isolates from ovine subclinical mastitis as *Staph. epidermidis*.

This is the first report of speciation of CNS from confirmed cases of ovine mastitis. Of the 41 isolates studied, 80% were speciated by the 'API-Staph SYSTEM' and 90% by a combination of biochemical tests. Forty (98%) isolates were speciated by one of the two methods and for 28 (68%) there was agreement between the two methods. The majority of recognized staphylococcal species is

Table 3. *Allocation of the isolates into one of the groups:* hyicus-simulans (*group A*), xylosus (*group B*), *of other species* (*group C*)

	'API-Staph SYSTEM'	Biochemical tests
Isolates from acute clinical mastitis		
1, 2, 8, 12, 13, 17, 19	group A	group A
3, 5, 7	group C	group C
4, 6, 11, 14, 15, 16, 18	group B	group B
9, 10	group C	group A
Isolates from subclinical mastitis		
20, 21, 24, 26, 27, 28, 29, 30, 33, 34, 35, 36	group C	group C
22, 23, 25	group A	group A
31, 32	group B	group B
Isolates from chronic clinical mastitis		
37, 38, 39, 40, 41	group A	group A

of human origin; this might explain why some isolates could not be speciated and why the two methods employed for speciation gave different results for some isolates.

Using the results of the two systems of identification, it is also possible to place the isolates into one of three groups, namely: a *hyicus-simulans* group (group A), a *xylosus* group (group B) and a group of other species (group C) (Table 3). Fermentation of arabinose, maltose and xylose and resistance to novobiocin would be useful properties to discriminate between the three groups.

The majority of isolates from acute clinical mastitis would be in group A or B, while the majority of those from subclinical mastitis would be in group C. All five isolates from chronic clinical mastitis would be in group A.

Staph. simulans, *Staph. xylosus* and *Staph. chromogenes* (or *Staph. hyicus* using the combination of biochemical tests) were isolated from clinical and subclinical mastitis; *Staph. simulans* and *Staph. xylosus* were also isolated from the teat skin and teat canal of healthy ewes [3]. Hence, it is evident that CNS are associated with the ovine mammary gland in health and disease. Mastitis has been induced by the intramammary inoculation of *Staph. simulans*, *Staph. xylosus* and *Staph. chromogenes* [16, 17]. However, differences were observed in the pathogenicity of these three species for the ovine [16] and murine [3, 18] mammary gland, although they had all been initially isolated from mastitis.

The antibiotic resistance pattern of *Staph xylosus*, *Staph. simulans*, *Staph. hyicus* and *Staph. epidermidis* was similar for isolates of each of these species and independent of the origin of the isolates (from clinical or from subclinical mastitis). Therefore, the antibiotic resistance, which seems to be associated with species, may be used as a further characteristic in the speciation of staphylococci.

Coagulase-negative staphylococci, whose aetiological role in mastitis is established, deserve further study, particularly in relation to their epidemiology and pathogenesis.

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