

From the Institute of Medical Microbiology, University of Lund,
Sweden.

COAGULASE-NEGATIVE, NOVOBIOCIN- RESISTANT STAPHYLOCOCCI ON THE SKIN OF ANIMALS AND MAN, ON MEAT AND IN MILK*

By

*Per-Anders Mårdh, Birgitta Hovelius, Kenneth Hovelius**
and Per Olof Nilsson****

PER-ANDERS MARDH, BIRGITTA HOVELIUS, KENNETH HOVELIUS and PER OLOF NILSSON: *Coagulase-negative, novobiocin-resistant staphylococci on the skin of animals and man, on meat and in milk.* Acta vet. scand. 1978, 19, 243—253. — The occurrence of coagulase-negative, novobiocin-resistant staphylococci, i.e. *Staphylococcus cohnii*, *Staphylococcus saprophyticus*, *Staphylococcus sciuri* and *Staphylococcus xylosus*, on the skin of animals and man has been studied. On cultures from cats, cows, dogs, guinea pigs, mice, rabbits and sheep studied, such organisms were predominant among the coagulase-negative staphylococci. From the skin of the hands of 21 of 38 persons whose professions brought them into contact with animals, e.g. inseminators, slaughterhouse workers and veterinarians, coagulase-negative, novobiocin-resistant staphylococci were isolated. This finding contrasted with that regarding 50 persons lacking such contacts, of whom only 1 harboured such bacteria. *S. saprophyticus* was isolated only from those slaughterers presenting with wounds on their hands. Coagulase-negative, novobiocin-resistant staphylococci were also isolated from every second specimen collected from the surface of meat at a slaughterhouse. No difference in the culture results could be demonstrated from specimens collected before and after cutting-up of the carcasses. Of 26 strains of coagulase-negative, DNase-negative staphylococci isolated from milk with pathological CMT, all but 5 were novobiocin-resistant. Fifteen were classified as *S. xylosus*, 4 as *S. sciuri* and 1 as *S. cohnii*. Of another 15 DNase-positive strains, 3 were resistant to novobiocin. Finally, clinical infections with coagulase-negative, novobiocin-resistant staphylococci in man, e.g. urinary tract infections caused by *S. saprophyticus*, are considered in relation to possible contagious reservoirs and modes of spread.

staphylococci; coagulase-negative; novobiocin-resistant; skin flora; animals; meat; milk.

* This study was supported by a grant from The Swedish Medical Research Council.

** District veterinary officer, Falkenberg, Sweden.

*** Chief veterinary officer, Swedish Laboratory Services Ltd., Kalmar, Sweden.

The coagulase-negative staphylococci constitute a heterogeneous group of bacteria, the species differentiation of which is still controversial (*Subcommittee 1976*). These organisms can be divided into 2 groups on the basis of their susceptibility to novobiocin. The Subcommittee on Taxonomy of Staphylococci and Micrococci has recommended that the coagulase-negative, novobiocin-resistant staphylococci should be differentiated into 3 species, i.e. *Staphylococcus cohnii*, *Staphylococcus saprophyticus* and *Staphylococcus xylosus* (*Subcommittee 1976*). Recently, the recognition of a fourth species has been proposed, i.e. *Staphylococcus sciuri* (*Kloos et al. 1976*).

Coagulase-negative staphylococci have been recognized as aetiological agents of mastitis in cows and goats (*Holmberg 1973*), of urinary tract infections (UTI) in dogs (*Clark 1974*) and of exudative epidermitis in pigs (*Ødegaard & Fossum 1966*). In most investigations on coagulase-negative staphylococci in animals and dairy products, novobiocin-resistant strains seem to have been misdiagnosed as micrococci and, because such organisms have not been considered pathogenic, have not been subjected to further study.

In man, clinical infections by coagulase-negative, novobiocin-sensitive staphylococci have been recognized with increasing frequency, particularly in the compromised host. Like *Staphylococcus aureus*, coagulase-negative staphylococci can produce enterotoxin (*Danielsson & Hellberg 1977*). Coagulase-negative, novobiocin-resistant staphylococci, i.e. *S. saprophyticus*, are known to cause UTI, particularly in young woman. The organism has also been demonstrated in urine collected from the renal pelvis of patients with nephrolithiasis (*Hovelius et al. in press*).

In the present study the occurrence of coagulase-negative, novobiocin-resistant staphylococci on the skin of animals and on the hands of persons having/not having contact with animals in their daily work was studied. The contamination of meat at the abattoir by such staphylococci was studied, as was the presence of coagulase-negative, novobiocin-resistant staphylococci in milk from cows with and without clinical signs of mastitis.

MATERIALS AND METHODS

Animals. Animals of the following species were studied: 6 cats, 12 cows, 4 dogs, 2 goats, 6 horses and 5 pigs. With few exceptions, the animals of each species came from different farms.

In addition, guinea pigs, mice and rabbits, 5 of each, from a medical research laboratory were studied.

Humans. Thirty-eight persons having professional contact with animals, i.e. inseminators, slaughterhouse workers, veterinarians, and 50 persons without such contacts in their daily work, i.e. staff in a hospital microbiological laboratory, were studied. Thirty-five of the laboratory staff were females as were all inseminators, while the remaining persons studied were males.

Sampling from animals and humans. After the persons had washed their hands under running tap water, specimens were collected by rubbing a cotton-tipped swab on the palms and the backs of the hands. Sampling was done during working hours. From the animals, specimens were collected by rubbing a cotton-tipped swab on the skin on the back. The specimens from both animals and humans were transported in a modified Stuart medium (Gästrin & Kallings 1968).

Meat and meat products. From the surface of meat from 20 pigs and 20 calves, small pieces were cut off immediately after slaughter, a procedure which was repeated after cutting-up. The specimens were collected by sterile means, transferred to tryptone broth (Oxoid), pH 7.2, and so transported to the laboratory.

Staphylococci isolated from milk. Thirty strains of coagulase-negative staphylococci, 15 DNase-positive and 14 DNase-negative, isolated from milk of cows without clinical signs of mastitis, but with a pathological California Mastitis Test (CMT) were studied. In addition, 12 DNase-negative strains from cows with clinical signs of mastitis were also studied. CMT varied between 2 and 5, corresponding to a total cell count of 0.15—5.0 $\times 10^6$ per ml milk.

Culture and identification techniques. The specimens were cultured on agar plates consisting of Blood agar base No. 2 (Oxoid) with 4 % defibrinated horse blood. The inoculated plates were incubated at 37°C for 18 hrs. Isolated staphylococci were tested for coagulase production. Coagulase-negative strains were tested for their susceptibility to novobiocin by disc diffusion tests (Ericson & Sherris 1971) using Wellco Susceptibility Test agar and paper discs containing 5 μ g novobiocin (AB Biodisk, Stockholm). The strains were considered "resistant" to novobiocin when the inhibitory zone was < 15 mm in diameter. This corresponds to a MIC of > 1 μ g/ml. All the staphylococcal strains

studied were also tested on an agar medium containing glycerol and erythromycin according to *Schleifer & Kloos (1975)*. The staphylococcal strains were also tested for their ability to ferment glucose (*Subcommittee 1965*), and for arginine dihydro-lase activity (*Digranes & Oeding 1975*). All coagulase-negative, novobiocin-resistant strains were tested for their ability to ferment arabinose, cellobiose, maltose, mannitol, sucrose, trehalose and xylitol. The tests were made on solid medium, which was incubated aerobically (*Kloos et al. 1974*).

RESULTS

Animals

On the cultures of the specimens cultured from the skin of all the animals studied, coagulase-negative, novobiocin-resistant strains predominated among the staphylococci isolated (Table 1).

Table 1. Isolation of coagulase-negative, novobiocin-resistant staphylococci from the back of animals.

Animal	Number of animals studied	Number of animals with growth of				
		<i>S. cohnii</i>	<i>S. saprophyticus</i>	<i>S. sciuri</i>	<i>S. xylosus</i>	NT*
Cat	6	1	2		2	3
Cow	12	4	4	1	2	3
Dog	4		1		3	
Goat	2		1	2	1	
Guinea pig	5	1		3	4	
Horse	8	1	1		2	4
Mouse	7	1	1	2	2	
Pig	5	1		1	2	1
Rabbit	5	1	2		1	

* Coagulase-negative, novobiocin-resistant staphylococci, which could not be identified as to species when using the criteria of the *Subcommittee (1976)*.

Generally speaking, staphylococci of 1 or more of the 4 novobiocin-resistant species, i.e. *S. cohnii*, *S. saprophyticus*, *S. sciuri* and *S. xylosus*, were isolated. No obvious variation in the occurrence of various staphylococcal species could be demonstrated among the animals studied.

Humans

On the hands of 21 of the 38 persons who had contact with animals in their professions, novobiocin-resistant staphylococci

were isolated. Of the 31 slaughterhouse workers investigated, 17 had wounds on their hands. It is notable that *S. saprophyticus* was isolated from 13 of these 17 slaughterhouse workers, but from none of the 14 lacking such wounds.

S. saprophyticus could be isolated from only 1 of those persons studied who did not have contact with animals in their daily work — a woman working in a hospital bacteriological laboratory dealing exclusively with urine specimens. All other staphylococcal strains isolated from the laboratory staff were sensitive to novobiocin (Table 2).

Table 2. Isolation of coagulase-negative, novobiocin-resistant staphylococci from the hands of persons having/not having contact with animals in their work.

Group of persons	Number of persons studied	Number of persons with growth of				
		<i>S. cohnii</i>	<i>S. saprophyticus</i>	<i>S. sciuri</i>	<i>S. xylosus</i>	NT*
Inseminators	4	1	2	0	2	0
Slaughterhouse workers	31	3	13	2	2	4
Veterinarians	3	0	1	3	2	1
Laboratory staff	50	0	1	0	0	0

* See footnote to Table 1.

Meat

Coagulase-negative, novobiocin-resistant staphylococci were recovered from about half of all meat specimens collected at the abattoir (Table 3). No differences in this respect could be demonstrated between specimens taken immediately after slaughter and those taken after the cutting-up.

Table 3. Coagulase-negative, novobiocin-resistant staphylococci isolated from beef and pork. Specimens collected from the surfaces of the meat after slaughter.

	Number of animals studied	Number of specimens with growth of				
		<i>S. cohnii</i>	<i>S. saprophyticus</i>	<i>S. sciuri</i>	<i>S. xylosus</i>	NT*
Beef	20	1	7	2	1	3
Pork	20	0	4	3	0	3

* See footnote to Table 1.

Milk

The classification of the coagulase-negative staphylococci isolated from milk specimens with pathological CMT is shown in Table 4. Three of the DNase-positive strains were resistant to

Table 4. Classification of 15 DNase-positive and 14 DNase-negative, coagulase-negative staphylococcal strains isolated from milk specimens with pathological California Mastitis Test.

Organism	DNase-positive	DNase-negative
<i>S. saprophyticus</i>	1*	0
<i>S. sciuri</i>	0	3
<i>S. xylosus</i>	0	7
Non-typable, novobiocin-resistant staphylococci	2	1
Novobiocin-sensitive staphylococci	12	3

* DNase-reaction weak.

novobiocin and 1 strains could be classified as *S. saprophyticus*. Among the 14 DNase-negative strains tested, 12 were novobiocin-resistant, 7 of which were classified as *S. xylosus*, while 3 strains were identified as *S. sciuri*. Of the 12 DNase-negative staphylococcal strains studied, which were isolated from cows with clinical signs of mastitis, 10 belonged to novobiocin-resistant species; *S. xylosus* in 8 and *S. sciuri* and *S. cohnii* in 1 instance each, respectively.

DISCUSSION

The family Micrococcaceae consists of gram-positive, catalase-positive cocci, including the 2 genera *Micrococcus* and *Staphylococcus*. The bacteria of genus *Staphylococcus* have a lower guanine-cytosine (GC) DNA content than the bacteria of genus *Micrococcus* (*Silvestri & Hill 1965*). The cell wall of the bacteria in these 2 genera differs in its chemical composition; teichoic acid is only found in bacteria of genus *Staphylococcus* (*Schleifer & Kandler 1972*).

Glucose fermentation tests were earlier used to distinguish micrococci from staphylococci (*Evans et al. 1955*). However, this test leads to a misclassification of novobiocin-resistant, coagulase-negative staphylococci as micrococci. For the differentiation of micrococci and staphylococci a test based on a combi-

nation of tests for glycerol fermentation and erythromycin susceptibility has recently been described (*Schleifer & Kloos 1975*). In our study, we found strains isolated from the skin flora of animals having a colony morphology with the same appearance as staphylococci and which would not grow or ferment glycerol on the glycerol-erythromycin-containing medium. Furthermore, these strains did not usually ferment the other carbohydrates used or at most only a few of them. In gram-stained smears they usually looked like "sarcina".

The present study has shown that coagulase-negative, novobiocin-resistant staphylococci can, as a rule, be isolated from the skin of domestic and laboratory animals. In these animals, such bacteria occur in larger numbers than they do in the human skin flora.

Coagulase-negative staphylococci resistant to novobiocin can also often be isolated from the skin of the hands of persons whose work brings them into contact with animals. From persons who did not have contact with animals in their daily work, such staphylococci were isolated only occasionally.

S. saprophyticus has sometimes been found to occur transiently and in low numbers on the skin of the arms and legs of man (*Kloos & Musselwhite 1975*). On the basis of findings in the present study it was not possible to assess whether *S. saprophyticus* found on the hands of inseminators, abattoir workers and veterinarians occurred only transiently as contaminants, or whether the bacteria had colonized their skin.

The finding that *S. saprophyticus* could be isolated exclusively from the skin of the hands of those slaughterers who presented with wounds, would appear to imply a true colonization rather than a mere contamination. However, further studies are necessary to establish whether *S. saprophyticus* can occur as a primary pathogen in wounds. The frequent finding of *S. saprophyticus* in pus from this group of persons stands in contrast to the highly infrequent finding of this organism in pus specimens obtained from a general population (*Hovelius & Mårdh 1977*).

Wounds on the hands are an occupational hazard for slaughterhouse workers and apart from being a nuisance in themselves, also constitute a potential risk for food poisoning; such wounds may be a contagious reservoir for enterotoxin-producing bacteria, as has been well documented in the case of *S. aureus* (*Untermann*

1972). Coagulase-negative staphylococci may also produce such toxins (Bergdoll *et al.* 1967). Food poisoning by coagulase-negative, toxin-producing staphylococci has been described by Breckinridge & Bergdoll (1971). Of the strains of *S. saprophyticus* isolated from the wounds of the slaughterers studied, 12 were tested for production of A, B, C₁ and E enterotoxin. However, none of the strains produced such toxins (Danielsson, Hovelius, Mårdh, unpublished data).

S. saprophyticus is a common cause of UTI in women in their early reproductive years. Nothing is known about the contagious reservoir or the mode of transmission of the causative organism. Such infections may be endogenous, since the organism can occur in the periurethral flora in healthy persons too (Mårdh *et al.* in press). Moreover, the possibility that such infections may be derived from animals or meat — both fresh and cured — can not be dismissed.

It is notable that in the few young men, lacking signs of obstructive uropathy, in whom we have diagnosed UTI with *S. saprophyticus*, the majority had jobs requiring contact with live animals or animal carcasses, e.g. slaughterhouse workers and sheep farmers.

S. saprophyticus isolated from urine specimens of males and females with UTI and from animals was often found to differ in its ability to ferment xylitol. The animal strains were usually xylitol-negative, whereas most human strains were xylitol-positive. Kloos & Schleifer (1975) found 29 % of 83 strains of *S. saprophyticus* isolated from human skin to be xylitol-negative. Thus, further studies are required to establish whether differences between animal and human strains of this staphylococcal species do exist.

It has been speculated that coagulase-negative staphylococci may be an aetiological agent of mastitis in cows and goats. Infections with coagulase-negative staphylococci are probably more common in subchronic than acute cases of mastitis (Holmberg 1973). Our study indicates that coagulase-negative, DNase-negative staphylococci isolated from milk with a pathological CMT are often novobiocin-resistant, as are some DNase-positive strains. We found that most novobiocin-resistant strains isolated from such milk belonged to *S. xylosus*, while 1 strain, which showed a weak DNase reaction, was classified as *S. saprophyticus*.

In studies on staphylococci isolated from milk from cows

with mastitis, the bacteria have been classified according to *Baird-Parker* (1963). Use of this classification may lead to misclassification of coagulase-negative, novobiocin-resistant staphylococci as micrococci, which could explain why in this context such staphylococci have not been recognized earlier. Micrococci have generally been regarded as not being of clinical importance.

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SAMMANFATTNING

Koagulasnegativa, novobiocinresistenta stafylokokker på huden hos djur och människa samt i kött och mjölk.

Förekomst av koagulasnegativa, novobiocinresistenta stafylokokker, d.v.s. *Staphylococcus cohnii*, *Staphylococcus saprophyticus*, *Staphylococcus sciuri* och *Staphylococcus xylosus*, på huden hos djur och människa har studerats. I odlingar från katt, ko, hund, marsvin, mus och kanin var dessa organismer vanligt förekommande. Från huden på händerna hos 21 av 38 personer med yrken som medför kontakt med djur, såsom seminatórer, slakteripersonal och veterinärer, isolerades koagulasnegativa, novobiocinresistenta stafylokokker.

Av 50 personer som saknade sådan djurkontakt, förekom däremot sådana bakterier hos endast en person. Koagulasnegativa, novobiocinresistenta stafylokker kunde isoleras från vartannat prov taget från ytan av kött på ett slakteri. Ingen skillnad kunde påvisas i odlingsresultat från köttprov tagna före och efter styckning. Av 26 koagulasnegativa, DNas-negativa stafylokockstammar isolerade från mjölk med patologiskt CMT-värde var 21 novobiocinresistenta. Femton klassificerades som *S. xylosus*, fyra som *S. sciuri* och en som *S. cohnii*. Av 15 koagulasnegativa, DNas-positiva stammar från mjölkprov var tre resistenta mot novobiocin.

(Received December 12, 1977).

Reprints may be requested from: Doc. P.-A. Mårdh, Institute of Medical Microbiology, Sölvegatan 23, S-223 62 Lund, Sweden.