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P. Trinidad  
*LSU Agricultural Center*

S. C. Nickerson  
*LSU Agricultural Center*

D. G. Luther  
*LSU Agricultural Center*

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# Antimicrobial Susceptibilities of Staphylococcal Species Isolated from Mammary Glands of Unbred and Primigravid Dairy Heifers<sup>1</sup>

P. TRINIDAD,<sup>2</sup> S. C. NICKERSON, and D. G. LUTHER<sup>3</sup>

Mastitis Research Laboratory  
Hill Farm Research Station  
Louisiana Agricultural Experiment Station  
Louisiana State University Agricultural Center  
Route 1, Box 10  
Homer 71040

## ABSTRACT

Staphylococcal isolates from teat canal keratin and mammary secretion samples of unbred and primigravid Jersey heifers were tested in vitro for susceptibility to 12 antimicrobial agents. More than 92% of the 311 isolates were susceptible to all antimicrobial agents tested. Staphylococci other than *Staphylococcus aureus* demonstrated an overall susceptibility of 98.3% to all antibiotics, and *Staphylococcus aureus* demonstrated a 97% susceptibility. Across all staphylococcal species, susceptibility of isolates from secretion samples was 98.1%, and susceptibility of isolates from teat canal keratin samples was 93.1%. Differences in susceptibilities were observed among herds.

(Key words: antibiotic susceptibility, heifers, staphylococci)

## INTRODUCTION

Antimicrobial therapy plays an important role in a mastitis control program. Two of the five recommended mastitis control practices are based on therapy and include prompt treatment of clinical mastitis (7, 8).

Successful treatment of intramammary infections (IMI) often depends on determining susceptibility of the etiological agent to antimicro-

bial drugs. Several reports (4, 10, 11, 16) have been published regarding susceptibility patterns on isolates from infected mammary glands of lactating cows. Other reports (10, 11, 16) have dealt with susceptibility of specific staphylococci isolated from lactating mammary glands to certain antimicrobial agents. In addition, variation in susceptibility patterns to antimicrobial agents among herds has been documented (9, 15); however, reports on susceptibility of isolates from heifer mammary glands have not been published.

Recent work on prevalence of mastitis in unbred and primigravid heifers demonstrated that IMI were present in 96.9% of animals and 74.6% of quarters (17). Likewise, teat canal colonizations were present in 93.1% of heifers and 70.7% of quarters. The majority of IMI and teat canal colonizations were caused by *Staphylococcus aureus* and *Staphylococcus* spp. The secretory tissue in mammary glands of heifers could be compromised at an early age due to such infections, which could adversely affect future milk yield.

Currently, there are no management techniques specifically designed for control of mastitis in heifers. Such procedures are warranted. Determining susceptibility patterns would aid in selection of antibiotics to treat dairy heifers prior to first parturition.

The objective of this study was to determine antimicrobial susceptibilities of staphylococcal species isolated from teat canal keratin and mammary secretions of unbred and primigravid dairy heifers.

## MATERIALS AND METHODS

### Isolates

Samples of teat canal keratin and mammary secretion were collected from 85 unbred and

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<sup>2</sup>University of Puerto Rico, Mayaguez Campus, Department of Animal Science, PO Box 5000, Mayaguez, PR 00709.

<sup>3</sup>Department of Veterinary Science, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803.

primigravid Jersey heifers from two commercial dairy herds and one research herd. Prior to collecting teat canal keratin samples, each teat meatus was scrubbed with a cotton pledget moistened with 70% ethyl alcohol. A sterile calcium alginate swab on an ultrafine flexible aluminum shaft (Calgiswab, Type A: Spectrum Laboratories, Inc., Houston, TX) was inserted approximately 2 mm into the teat canal and rotated gently against the wall of the canal. After removal, the swab was placed in .5 ml of sterile physiological saline, vortexed, and .1 ml was pipetted onto trypticase soy blood agar (TBA) (BBL Microbiology Systems, Cockeysville, MD) plates containing .1% esculin (Sigma Chemical Co., St. Louis, MO) and 5% calf blood. The sample was spread evenly across the medium surface using an angled glass rod. Plates were incubated at 37°C for 48 h.

Secretion samples were collected and processed as recommended by the National Mastitis Council (2). Presumptive identification to genus was as described (2). Briefly, a .01-ml aliquot of secretion was plated on TBA and incubated at 37°C for 48 h. Gram stain, colony morphology, hemolytic pattern, and catalase production were used to delineate isolates into the genus *Staphylococcus*. Identification of species was performed with the Staph Trac system (Analytab Products, Plainville, NY). Staphylococci that could not be identified as a species were classified as *Staphylococcus* spp. Because of the limited number of isolates, the following species were grouped as other staphylococci: *Staphylococcus xylosus* (n = 4); *Staphylococcus epidermidis* (n = 1); *Staphylococcus simulans* (n = 1); *Staphylococcus saprophyticus* (n = 1); *Staphylococcus haemolyticus* (n = 1); *Staphylococcus warneri* (n = 1); *Staphylococcus hominis* (n = 6); *Staphylococcus capitis* (n = 2); *Staphylococcus cohnii* (n = 2); and *Staphylococcus* spp. (n = 6).

#### Antimicrobial Susceptibility Testing

Tests were performed following the standardized single high potency disk diffusion method recommended by the National Committee for Clinical Laboratory Standards (14). All isolates were tested for antibiotic susceptibility to the following 12 antimicrobial drugs (BBL Microbiology systems, Cockeysville, MD): am-

picillin (10 µg); amoxicillin plus clavulanic acid (30 µg); cephalothin (30 µg); erythromycin (15 µg); gentamicin (10 µg); novobiocin (30 µg); oxacillin (1 µg); penicillin (10 U); streptomycin (10 µg); sulfamethoxazole (23 µg) with trimethoprim (1.25 µg); tetracycline (30 µg); and vancomycin (30 µg).

Briefly, five isolated colonies were transferred from TBA to 5 ml of trypticase soy broth (BBL). The culture was incubated at 37°C until it reached a turbidity of .5 McFarland standard and was inoculated onto a 150 × 15 mm petri dish containing Mueller-Hinton agar (BBL). Antibiotic disks were applied, and plates were incubated at 35°C for 18 h. The diameters of zones of growth inhibition were measured in millimeters and reported as susceptible, intermediate, or resistant. The quality control organism, *S. aureus* ATCC 25923, was included in each trial.

#### Data Analysis

For purposes of analysis, the isolates were grouped as *S. aureus*, *S. chromogenes*, *S. hyicus*, and other staphylococci. Frequencies and percentages of susceptibilities were determined using SAS (SAS Institute Inc., Cary, NC).

#### RESULTS AND DISCUSSION

A total of 311 staphylococcal isolates from teat canal keratin and secretion samples was tested for susceptibility to 12 antimicrobial agents (Table 1). Strains tested for susceptibility were *S. aureus*, *S. chromogenes*, *S. hyicus*, and other staphylococci. Cephalothin and gentamicin were the only antimicrobial agents to which all isolates were 100% susceptible. More than 95% of all isolates were susceptible to the 12 antimicrobics except penicillin (92.3%) and ampicillin (92.3%). Owens and Watts (15) reported similar susceptibilities of staphylococci isolated from lactating cows to amoxicillin, cephalothin, gentamicin, sulfamethoxazole, and vancomycin. Likewise, McDonald and Anderson (12) reported similar results to cephalothin, erythromycin, gentamicin, and vancomycin in a study involving 813 staphylococcal isolates from IMI of lactating cows.

Overall susceptibility of *S. aureus* to the 12 antimicrobial agents was 97%, whereas that for *Staphylococcus* spp. was only slightly higher (98.3%). This is in agreement with McDonald

TABLE 1. Antibiotic susceptibilities of staphylococci isolated from teat canal keratin and mammary secretion of heifers to 12 antimicrobial agents.

Antibiotic	<i>S. aureus</i> (n = 48)	<i>S. chromogenes</i> (n = 167)	<i>S. hyicus</i> (n = 71)	Other staphylococci (n = 25)	Overall <sup>1</sup> (n = 311)
	(%)				
Ampicillin	97.1	88.6	98.6	88	92.3
Amoxicillin-clavulanic acid	95.8	100	100	96 (4) <sup>2</sup>	99
Cephalothin	100	100	100	100	100
Erythromycin	100	100	100	96 (4)	99.7
Gentamicin	100	100	100	100	100
Novobiocin	93.7 (6.3)	100	100	100	99
Oxacillin	100	100	100	96	99.7 (.3)
Penicillin	100	88.6	98.6	84	92.3
Streptomycin	77.1 (22.9)	99.4 (.6)	100	100	96.1 (3.9)
Sulfamethoxazole-trimethoprim	100	99.4 (.6)	100	100	99.7 (.3)
Tetracycline	100	100	97 (3)	100	99.7 (.3)
Vancomycin	100	99.7 (.3)	100	100	99.7 (.3)

<sup>1</sup>Includes all staphylococcal species.<sup>2</sup>Numbers in parentheses denote intermediate category.

and Anderson (10) who reported that *Staphylococcus* spp. isolated from lactating cows were more susceptible to antimicrobial agents than *S. aureus*. In this study, *S. aureus* was 100% susceptible to 8 of the 12 antimicrobics tested (Table 1). *Staphylococcus* spp. were 100% susceptible to cephalothin, gentamicin, and novobiocin. Similarly, Owens and Watts (15) reported 100% susceptibility of *Staphylococcus* spp. to the same antimicrobial agents. Likewise, McDonald and Anderson (12) reported 100% susceptibility of *Staphylococcus* spp. to gentamicin.

Penicillin is among the most commonly used antibiotics for treatment of mastitis in lactating and dry cows (8). Overall susceptibility of isolates to penicillin in this study was 92.3% (Table 1); reports on mature cows have ranged from 44.2% to 65% (9, 11, 12, 15, 16). Previous research (11, 12, 15, 16) was conducted with isolates collected from herds with mastitis problems, whereas results reported herein were based on isolates from unbred and primigravid heifers with no history of intramammary treatment. Thus, exposure to antimicrobial drugs

may account for differences in susceptibilities between heifers and cows.

*Staphylococcus aureus* is one of the most important pathogens causing mastitis (2, 3). In this study, 100% of *S. aureus* isolates were susceptible to penicillin (Table 1). Susceptibilities of *S. aureus* isolated from mammary glands of lactating cows ranged between 25.6 and 94% (4, 10, 11, 12, 15, 16). In a study conducted in Louisiana in 1969, Philpot (16) reported a 94% susceptibility of *S. aureus* from lactating cows to penicillin. This is in agreement with a 1987 study in Louisiana in which isolates from lactating cows of seven dairy herds demonstrated a 93% susceptibility to penicillin (15). The fact that *S. aureus* isolated from heifers was more susceptible than *S. aureus* isolated from lactating cows suggests that early treatment of heifers prior to first calving might be more efficacious.

*Staphylococcus chromogenes*, the most frequently isolated species in this study, exhibited 88.6% susceptibility to penicillin. *Staphylococcus hyicus*, the second most frequent isolate, exhibited a 98.6% susceptibility to penicillin.

TABLE 2. Antibiotic susceptibilities of staphylococci isolated from teat canal keratin samples (n = 165) of heifers to 12 antimicrobial agents.

Antibiotic	<i>S. aureus</i> (n = 21)	<i>S. chromogenes</i> (n = 89)	<i>S. hyicus</i> (n = 38)	Other staphylococci (n = 17)
	(%)			
Ampicillin	95.2	89.9	100	90.5
Amoxicillin-clavulanic acid	90.5	100	100	100
Cephalothin	100	100	100	100
Erythromycin	100	100	100	100
Gentamicin	100	100	100	100
Novobiocin	90.5 (9.5) <sup>1</sup>	100	100	100
Oxacillin	100	100	100	100
Penicillin	100	89.9	100	82.4
Streptomycin	71.4 (28.6)	100	100	100
Sulfamethoxazole-trimethoprim	100	98.9 (1.1)	100	100
Tetracycline	100	100	100	100
Vancomycin	100	98.9 (1.1)	100	100

<sup>1</sup>Numbers in parentheses denote intermediate category.

Susceptibilities of 39.7% for *S. chromogenes* and 66.4% for *S. hyicus* to penicillin have been reported for lactating cows (15). These results indicate that antibiotic therapy of the most common isolates in heifers could be more efficacious than in lactating cows.

Antibiotic susceptibilities of staphylococcal species isolated from teat canal keratin samples are presented in Table 2. Overall susceptibility to all antibiotics was 93.1%. *Staphylococcus aureus* and *S. chromogenes* were 100% susceptible to eight antibiotics, *S. hyicus* isolates were 100% susceptible to all antibiotics, and other staphylococci were 100% susceptible to nine antibiotics.

The teat canal plays an important role in prevention of mastitis (13). Microorganisms must traverse the tissues associated with the canal prior to entering and colonizing tissues within the udder. Despite inhibitory properties of teat canal keratin, bacteria survive in the canal and may produce metabolites that irritate secretory tissues (1, 6). Moreover, teat canal colonization could be a prelude to IMI (5). As a consequence, antibiotic treatment of the teat canal has been advocated by some workers (5, 6). However, susceptibilities of isolates from teat canal keratin have never been reported.

Susceptibilities of staphylococcal species isolated from mammary secretion samples to 12 antibiotics are in Table 3. Overall susceptibility was 98.1%. Staphylococci isolated from secretion samples were 100% susceptible to cephalothin, gentamicin, oxacillin, sulfamethoxazole-trimethoprim, and vancomycin. Owens and Watts (15) reported similar susceptibilities of isolates from lactating cows to the same antibiotics with the exception of oxacillin (60.3%). *Staphylococcus aureus* organisms were 100% susceptible to all but two of the antibiotics tested: novobiocin (96.3%) and streptomycin (81.5%). *Staphylococcus chromogenes* and *S. hyicus* were 100% susceptible to nine antibiotics, and other staphylococci were 100% susceptible to eight antibiotics. Owens and Watts (15) reported 100% susceptibilities for *S. aureus* to the same antibiotics with the exceptions of ampicillin, erythromycin, oxacillin, penicillin, and tetracycline.

Comparing overall susceptibilities, teat canal keratin isolates showed 93.1% susceptibility, whereas secretion isolates showed a slightly higher susceptibility (98.1%). Reasons for differences in susceptibilities obtained from the same animals are unknown. However, differences in bacterial environment, e.g., keratinac-

TABLE 3. Antibiotic susceptibilities of staphylococci isolated from mammary secretion samples (n = 146) of heifers to 12 antimicrobial agents.

Antibiotic	<i>S. aureus</i> (n = 27)	<i>S. chromogenes</i> (n = 78)	<i>S. hyicus</i> (n = 33)	Other staphylococci (n = 8)
			(%)	
Ampicillin	100	87.2	97	87.5
Amoxicillin-clavulanic acid	100	100	100	87.5
Cephalothin	100	100	100	100
Erythromycin	100	100	100	100
Gentamicin	100	100	100	100
Novobiocin	96.3	100	100	100
Oxacillin	100	100	100	100
Penicillin	100	87.2	97	87.5
Streptomycin	81.5	98.7 (1.3) <sup>1</sup>	100	100
Sulfamethoxazole-trimethoprim	100	100	100	100
Tetracycline	100	100	97 (3)	100
Vancomycin	100	100	100	100

<sup>1</sup>Numbers in parentheses denote intermediate category.

eous teat canal vs. intramammary fluid, may account for the difference. The fact that bacteria colonizing teat canal keratin are more drug resistant is of some concern because if they breach the canal and cause IMI, more drug-resistant infections may result and pose a threat to mammary secretory tissues.

Variability in susceptibility within and among herds was noted. This fact has been reported previously for lactating cows (6, 9, 12). Herd C demonstrated an overall susceptibility of 99.7% to all 12 drugs, and 100% of isolates were susceptible to amoxicillin-clavulanic acid, cephalothin, erythromycin, gentamicin, novobiocin, oxacillin, sulfamethoxazole-trimethoprim, tetracycline, and vancomycin. Isolates from Herd B had the lowest percentage of isolates that were susceptible to ampicillin (82.8%) and penicillin (82.8%). Although differences in susceptibility were observed among herds, no relationship between *S. aureus* and the other staphylococcal species was observed within herd.

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