Antimicrobial Susceptibility of *Staphylococcus saprophyticus* and Urethral Staphylococci

T. J. MARRIE^{1,2*} AND C. KWAN¹

Departments of Medicine¹ and Microbiology,² Dalhousie University, and Victoria General Hospital, Halifax, Nova Scotia B3H 2V8, Canada

Received 3 May 1982/Accepted 18 June 1982

The activity of eight antimicrobial agents was determined against 115 isolates of *Staphylococcus saprophyticus*. All were susceptible to ampicillin, cephalexin, and trimethoprim-sulfamethoxazole and resistant to nalidixic acid and novobiocin. A bimodal pattern of susceptibility to erythromycin was observed: 80% were inhibited by 0.25 μ g/ml, whereas 13% required \geq 128 μ g/ml. The following urethral staphylococci were susceptible to ampicillin, cephalexin, and nitrofurantoin but resistant to nalidixic acid: *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. warneri*, *S. simulans*, and *S. cohnii*.

Staphylococcus saprophyticus has now been shown to be an important cause of urinary tract infection in young adult females (1-4, 6, 8, 12). However, there are still very few data on the antimicrobial susceptibility of this microorganism (4, 5, 8, 9). We determined the susceptibility of S. saprophyticus to eight antimicrobial agents, and we also determined the activity of five of these agents against isolates of coagulasenegative staphylococci recovered from the urethras of healthy females.

MATERIALS AND METHODS

Bacteria. The isolates of S. saprophyticus were from the midstream urine specimens of 115 women with symptoms of a urinary tract infection. These were identified as S. saprophyticus by a modification (carbohydrate fermentation reactions were performed in broth rather than in agar) of the method of Kloos and Schleifer (7). The remaining species of staphylococci were isolated from urethral urine specimens obtained from healthy women of reproductive age as part of a study of urethral flora and from women with the urethral syndrome. These organisms were classified by species as outlined above. Only single isolates of each species from each person were tested.

The following control organisms were included with each run: S. aureus ATCC 25923, Escherichia coli ATCC 25922, S. saprophyticus ATCC 15305, and Oxford S. aureus.

Media and susceptibility tests. Organisms to be tested were inoculated into tryptose phosphate broth (Difco Laboratories, Detroit, Mich.) and incubated at 37°C for 6 h. Turbidity was adjusted with tryptose phosphate broth to that of one-half the no. 1 McFarland standard. An agar dilution susceptibility test was performed as described by Washington and Sutter (13). A Steers replicator (11) was used to inoculate the suspension to the Mueller-Hinton agar (GIBCO Diagnostics, Madison, Wis.). A plate of test medium without antibiotics was inoculated at the beginning and end of each series of tests to serve as a growth control. The plates were then incubated at 37°C for 18 h. The minimal inhibitory concentration was read as the lowest concentration of antimicrobial agent yielding no growth.

Antimicrobial agents. Laboratory standard powders were supplied as follows: ampicillin, Ayerst Laboratories, Montreal, Quebec, Canada; benzylpenicillin G, Glaxo Laboratories, Toronto, Ontario, Canada; trimethoprim lactate and sulfamethoxazole, Burroughs Wellcome Ltd., La Salle, Quebec, Canada; erythromycin, Abbott Laboratories, Montreal, Quebec, Canada; nalidixic acid, Winthrop Laboratories, Aurora, Ontario, Canada; and nitrofurantoin, Norwick-Eaton Pharmaceuticals, Paris, Ontario, Canada.

The sulfamethoxazole and nalidixic acid were dissolved in 0.1 N NaOH. The nitrofurantoin was dissolved in dimethylformamide and subsequently diluted in 25% dimethylformamide in water. All other antibiotic powders were diluted in water.

RESULTS AND DISCUSSION

All 115 isolates of *S. saprophyticus* were susceptible to ampicillin, cephalexin, and trimethoprim-sulfamethoxazole, and all were resistant to nalidixic acid and novobiocin (Table 1).

All urethral staphylococci except S. cohnii were susceptible to novobiocin (Table 2). S. cohnii and S. xylosus have previously been shown to be resistant to novobiocin (10).

S. cohnii has been isolated more frequently from urine specimens than has S. xylosus (5). We have not recovered S. cohnii from the urethras of 100 healthy young women (7a). These organisms then represent false-positives if resistance to the 5- μ g novobiocin disk is used as

396 MARRIE AND KWAN

Antibiotic	Minimal inhibitory concn (µg/ml)			
	50%	90%	Range	
Ampicillin	0.25	0.25	≤0.258	
Penicillin	0.125	0.125	≤0.125–4	
Cephalexin	4	4	1–8	
Nitrofurantoin	64	64	16-64	
Nalidixic acid	>256	>256		
Erythromycin	0.25	128	0.25->256	
Trimethoprim- sulfamethoxazole	0.25-4.75	0.5-9.5	≤0.125-2.5-2.375-47.5	
Novobiocin	16	32	16–32	

TABLE 1. Comparative activities of eight antimicrobial agents against 115 S. saprophyticus isolates

a presumptive test for the identification of S. saprophyticus (2).

Most isolates of S. saprophyticus were resistant to nitrofurantoin at a minimal inhibitory concentration of 64 μ g/ml. All of the other coagulase-negative staphylococci were susceptible to nitrofurantoin (Table 2). Of all S. saprophyticus isolates, 80% were inhibited by 0.25 μ g of erythromycin per ml, but 13% were highly resistant, requiring \geq 128 μ g/ml. All of the isolates of staphylococci from the urethras of healthy females (Table 2) were inhibited by

TABLE 2. Comparative activities of five antimicrobial agents against various species of coagulase-negative staphylococci isolated from the urethras of healthy females and from women with the urethral syndrome

Organism (no. of isolates)	Antibiotic	Minimal inhibitory concn (µg/ml)		
		50%	90%	Range
S. epidermidis (30)	Ampicillin	0.25	16	≤0.125–16
	Cephalexin	2	2	0.5-8
	Nitrofurantoin	16	16	16-32
	Nalidixic acid	64	64	32-128
	Novobiocin	0.125	0.125	≤0.125
S. hominis (25)	Ampicillin	0.125	2	≤0.125-2
	Cephalexin	2	4	1–16
	Nitrofurantoin	32	32	16-32
	Nalidixic acid	64	128	32->256
	Novobiocin	0.25	0.25	≤0.125–0.25
S. haemolyticus (16)	Ampicillin	0.25	1	≤0.125-2
	Cephalexin	1	2	2-4
	Nitrofurantoin	32	32	16-32
	Nalidixic acid	64	64	32-64
	Novobiocin	0.25	0.5	≤0.125–0.5
S. warneri (8)	Ampicillin	0.125	4	≤0.125-4
	Cephalexin	2	4	14
	Nitrofurantoin	32	32	16-32
	Nalidixic acid	128	128	32-128
	Novobiocin	0.125	0.25	≤0.125–0.5
S. simulans (7)	Ampicillin	0.125	0.125	≤0.125
	Cephalexin	4	4	2-4
	Nitrofurantoin	32	32	16-32
	Nalidixic acid	64	128	64–128
	Novobiocin	0.125	0.25	≤0.125–0.25
S. cohnii (4)	Ampicillin	0.125	0.25	≤0.125-0.25
	Cephalexin	4	4	2-4
	Nitrofurantoin	32	32	16-32
	Nalidixic acid	>256	>256	256->256
	Novobiocin	16	32	16-32

Vol. 22, 1982

concentrations of ampicillin and cephalexin achievable in the urine.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Department of National Health and Welfare, Canada, and by a grant-in-aid from Eli Lilly Canada Ltd., Toronto, Ontario, Canada.

LITERATURE CITED

- Anderson, J. D., A. M. Clarke, M. E. Anderson, J. L. Isaac-Renton, and M. G. McLoughlin. 1981. Urinary tract infections due to *Staphylococcus saprophyticus* biotype 3. Can. Med. Assoc. J. 124:415-418.
- Digranes, A., and P. Jeding. 1975. Characterization of Micrococcaceae from the urinary tract. Acta Pathol. Microbiol. Scand. Sect. B 83:373-381.
- Hovelius, B., P. A. Mardh, and P. Bygren. 1979. Urinary tract infections caused by *Staphylococcus saprophyticus*. Recurrences and complications. J. Urol. 122:645-647.
- Hovelius, B., I. Thelin, and P. Mardh. 1979. Staphylococcus saprophyticus in the aetiology of nongonococcal urethritis. Br. J. Vener. Dis. 55:369-374.
- John, J. F., Jr., P. K. Gramling, and N. M. O'Dell. 1978. Species identification of coagulase-negative staphylococci from urinary tract isolates. J. Clin. Microbiol. 8:435-437.
- Jordan, P. A., A. Iravani, G. A. Richard, and H. Baer. 1980. Urinary tract infection caused by *Staphylococcus* saprophyticus. J. Infect. Dis. 142:510-515.
- 7. Kloos, W. E., and K. H. Schleifer. 1975. Simplified

scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. 1:82-88.

- 7a.Marrie, T. J., C. Kwan, M. A. Noble, A. West, and L. Duffield. 1982. Staphylococcus saprophyticus, as a cause of urinary tract infections. J. Clin. Microbiol. 16:427-431.
- Meers, P. D., W. Whyte, and G. Sandys. 1975. Coagulasenegative staphylococci and micrococci in urinary tract infections. J. Clin. Pathol. 28:270–273.
- Richardson, J. F., and R. R. Marples. 1980. Differences in antibiotic susceptibility between *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. J. Antimicrob. Chemother. 6:499-510.
- Schleifer, K. H., and W. E. Kloos. 1975. Isolation and characterization of staphylococcci from human skin. I. Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii, Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. Int. J. Syst. Bacteriol. 25:50-61.
- Steers, E., E. L. Foltz, and B. S. Graves. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. (Basel) 9:307-311.
- 12. Walmark, G., I. Anemark, and B. Telander. 1978. Staphylococcus saprophyticus: a frequent cause of urinary tract infections among female outpatients. J. Infect. Dis. 138:791-797.
- Washington, J. A., II, and V. L. Sutter. 1980. Dilution susceptibility test: agar and macro-broth dilution procedures, p. 453-458. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.