JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1975, p. 256-261

Vol. 1, No. 3
Printed in U.S.A.

Virulence Factors of Biotypes of *Staphylococcus epidermidis* from Clinical Sources

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Received for publication 19 November 1974

The biotyping scheme of Baird-Parker was applied to cultures of *Staphylococcus epidermidis* from patients. In all, 63.6% of 228 cultures belonged to biotype 1, followed by biotypes 4, 3, and 2 in decreasing order of incidence. When classified according to clinical source of isolation, cultures of *S. epidermidis* were most frequently isolated from urine, with 39.5% of 228 cultures from this source. Each of the four biotypes was distributed throughout all nine catagories of clinical sources. The production of virulence factors was based on the results of three groups of tests: (i) deoxyribonuclease, urease, gelatinase, caseinase, and lysozyme production; (ii) lipolytic activity on the tweens; and (iii) hemolysin production. Enzymatic activity was highest for organisms in biotype 1, followed by biotypes 3, 4, and 2 in decreasing order. Of the 228 cultures, 76.3% were lysed by lysostaphin. Resistance to antibiotics was highest for tetracycline, ampicillin, and penicillin, with rates of 54.8, 69.3, and 81.6%, respectively. The role of *S. epidermidis* as an etiological agent was studied by analyzing the laboratory and clinical data of 80 patients selected at random with bacteriuric *S. epidermidis*. Organisms in biotype 1 were most commonly associated with urinary tract infection. The significance of certain biotypes of *S. epidermidis* as opportunistic pathogens among compromised hosts in a hospital environment is discussed.

The role of *Staphylococcus epidermidis* in severe infections involving the colonization of prosthetic devices is well established (9, 21). However, the ubiquitous nature of this organism and our inability to distinguish potentially pathogenic strains make its etiological role in less severe infections difficult to assess. Because of its resistance to antibiotics (12), treatment of infections by *S. epidermidis* can present therapeutic problems (21). To date, epidemiological studies by bacteriophage typing of *S. epidermidis* have not been successful in our laboratory (unpublished data) or by others in this country (13). The most generally accepted method to distinguish subgroups of *S. epidermidis* is the biotyping scheme of Baird-Parker, with cultures primarily from nonclinical sources (4). The purpose of this study was to apply this typing scheme to *S. epidermidis* from patients and to correlate biotype with clinical source, resistance to antibiotics, and the production of virulence factors. Also, for one category of isolates, laboratory and clinical data of patients were analyzed to determine whether certain biotypes of *S. epidermidis* play a more prominent role in the etiology of urinary tract infections.

(Presented at the 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1974, San Francisco, Calif.).

MATERIALS AND METHODS

Cultures. A total of 228 cultures of *S. epidermidis* was obtained from the clinical laboratory of the University of Missouri Medical Center, Columbia, over a 9-month period, and each was classified as to its site of isolation. Identification of each culture as *S. epidermidis* was based on the results of four tests: gram-positive staining reaction, catalase production, anaerobic fermentation of glucose, and the inability to produce coagulase with human plasma. All cultures were stored at 4 C on brain heart infusion agar (BBL). Viability was maintained by subculture every 4 weeks.

Biochemical tests. Anaerobic fermentation of glucose and mannitol and acid produced aerobically from lactose and maltose were determined according to the method of Bentley et al. (8). Reactions were read at intervals of 1, 3, 5, and 10 days. Acid production from mannitol under aerobic conditions was determined with mannitol salt agar containing phenol red broth base (Difco), 1.5% agar (BBL), 1% D-mannitol, and 7.5% NaCl. Reactions were read after incubation for 18 to 24 h at 37 C. The production of acetoin (4),

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urease (22), gelatinase (8), and caseinase (8) was determined as described by others. Phosphatase activity was determined according to the procedure of Baird-Parker (4); however, the basal medium consisted of tryptose blood agar base (Difco). Deoxyribonuclease (DNase) production was determined with DNase test medium (Difco). No attempt was made to determine the thermal stability of the DNase produced (11). The production of lysozyme was determined after incubation for 3 days at 37 C under anaerobic conditions (9) on plates containing heart infusion agar (Difco) with lysozyme substrate (Difco) at a concentration of 1 mg/ml. The formation of a lytic zone around each colony was interpreted as a positive test. Lipolytic activity was determined on Sierra medium according to the procedure of Alder et al. (1). Tweens 20, 40, 60, and 80 (Sigma Chemical Co., St. Louis, Mo.) were the lipid substrates and were added to the basal medium, which was adjusted to pH 7.8.

Hemolysins. Determination of the production of alpha-, beta-, and delta-hemolysins was with human, sheep, and rabbit erythrocytes which had been washed three times with phosphate-saline buffer (pH 7.2) and added to heart infusion agar to a final concentration of 5%. Plates containing each type of blood were inoculated by radial streaks with cultures which had been grown overnight on brain heart infusion agar. Incubation was at 37 C for 18 to 24 h under increased (10%) CO_2 and again at 37 C for another 18 to 24 h under aerobic conditions. After each period of incubation, plates were read for hemolysis. To distinguish between alpha- and delta-hemolysins, the cross-streaking technique with a beta-lysin-producing strain of *Staphylococcus aureus* was employed (10).

Lysostaphin susceptibility. A modification of the procedure of Lachica et al. (11) was used for the detection of lysostaphin susceptibility with cultures grown overnight in tryptic soy broth (Difco). Lysostaphin (Schwarz/Mann Research Laboratory, Orangeburg, N.Y.) was dissolved in 0.1 M tris(hydroxymethyl)aminomethane buffer (pH 7.5) in a 0.15 M NaCl solution to give a final concentration of 33 μg/ml upon addition to broth cultures. A 0.1-ml quantity of lysostaphin-buffer solution or 0.1 ml of buffer was added to duplicate tubes containing 0.4 ml of tryptic soy broth culture. Both tubes were then incubated in a water bath at 37 C and read for lysis after 30-min and 1-h intervals. The test was recorded as negative if no clearing occurred after 3 h.

Antibiograms. Antibiograms were determined by the Kirby-Bauer method (7) with the following antibiotic disks: streptomycin, kanamycin, chloramphenicol, erythromycin, tetracycline, and penicillin G (Difco); cephalothin, ampicillin, methicillin (BBL); and lincomycin (Upjohn).

RESULTS

The results (not shown) of acid production from lactose, maltose, and mannitol under aerobic conditions, and the production of acetoin and phosphatase, were used to classify each culture into one of the four biotypes of Baird-Parker (5, 6). Three cultures could not be classified as to biotype, although each was identified as *S. epidermidis* based on their inability to produce coagulase and ferment mannitol anaerobically. The distribution of cultures based on biotype classification and source of isolation is shown in Table 1. In all, 145 (63.6%) of 228 cultures belonged to biotype 1, whereas only four (1.8%) of 228 cultures were in biotype 2. When classified according to clinical source of isolation, cultures of *S. epidermidis* were most frequently isolated from urine, with 90 (39.5%) of 228 cultures isolated from this source. Each of the four biotypes was distributed throughout all nine categories of clinical sources (Table 1).

A summary of the enzymatic activities of the cultures based on their biotype classification is shown in Table 2. Enzymatic activity was based on the results of three groups of tests. The first group consisted of tests for DNase, urease, gelatinase, caseinase, and lysozyme production; the second for lipolytic activity on the tweens; and the third for alpha-, beta-, and delta-hemolysin production. The average of individual positive reactions by cultures in each biotype for each group of tests was determined. Although individual differences were noted, enzymatic activity in the three groups of tests was highest for cultures in biotype 1. Cultures in biotype 2 were the least enzymatically active. The percentage of susceptibility to lysis by lysostaphin for cultures in each biotype was: biotype 1, 77.9; biotype 2, 25.0; biotype 3, 76.7; and biotype 4, 80.4. In all, 174 (76.3%) of the 228 cultures were lysed by this enzyme.

Table 3 lists a summary of the enzymatic activities of the cultures when classified according to clinical source of isolation. Although individual differences were observed, the enzymatic activities of the isolates from the differ-

TABLE 1. *Number and biotype of 228 cultures of S. epidermidis from clinical sources*

Clinical source	No. of cultures in biotype:				
	1	2	3	4	Unclassified
Urine	55	1	10	23	1
Wound	19	1	2	5	0
Abscess	13	1	6	1	0
Urogenital	13	0	2	3	0
Blood	6	0	7	3	1
Nasopharyngeal	15	0	0	2	0
Skin	11	0	1	2	0
Eye	3	0	2	3	0
Miscellaneous	10	1	0	4	1

ent sources were relatively uniform. Susceptibility to lysis by lysostaphin was highest for eye isolates; seven (87.5%) of eight cultures lysed, whereas only 12 (57.1%) of 21 cultures from abscesses lysed (not shown).

The percentages of cultures resistant to each of 10 antibiotics are shown in Table 4. Resistance was highest for the antibiotics tetracycline, ampicillin, and penicillin G, with rates of 54.8, 69.3, and 81.6%, respectively. On the other hand, resistance was lowest for the antibiotics chloramphenicol and cephalothin, with rates of 3.9 and 1.8%, respectively.

During the period in which this study was conducted, approximately 7% of the urine specimens collected from patients were found to

TABLE 2. *Summary[a] of enzymatic activities of 228 cultures of S. epidermidis according to biotype*

Test	% Positive reactions for cultures in biotype:			
	1(145)[b]	2(4)	3(30)	4(46)
DNase, urease, gelatinase, casinease, and lysozyme	53.5[c]	10.0	40.0	26.5
Lipolysis of tweens 20, 40, 60, and 80	64.7	56.3	51.7	47.8
Hemolysis by alpha-, beta-, and delta-hemolysin	23.6	12.5	20.9	22.3

[a] Individual reactions of tests in each group available upon request.
[b] Number in parentheses indicates number of cultures in biotype.
[c] Average of individual positive reactions within each group of tests.

TABLE 3. *Summary of enzymatic activities of 228 cultures of S. epidermidis according to clinical source*

Test	% Positive reactions for cultures from:[a]								
	Urine (90)[b]	Wound (27)	Blood (17)	NP (17)	Abscess (21)	UG (18)	Skin (14)	Eye (8)	Miscellaneous (16)
DNase, urease, gelatinase, caseinase, and lysozyme	42.9[c]	48.9	54.1	51.8	39.1	48.9	47.1	42.5	45.0
Lipolysis of tweens 20, 40, 60, and 80	54.5	63.9	53.0	72.1	67.9	73.6	57.1	50.0	50.0
Hemolysis by alpha-, beta-, and delta-hemolysin	23.1	24.1	23.5	22.1	20.3	23.6	19.6	25.0	20.3

[a] NP, Nasopharyngeal; UG, urogenital.
[b] Number in parentheses indicates number of cultures in source.
[c] Average of individual positive reactions within each group of tests.

TABLE 4. *Antibiotic resistance of 228 cultures of S. epidermidis according to biotype*

Antibiotic	% Resistant cultures in biotype:				
	1 (145)[a]	2 (4)	3 (30)	4 (46)	Total[b]
Streptomycin	20.7	25	20.0	21.7	20.6
Kanamycin	15.2	0	16.7	23.9	17.1
Chloramphenicol	3.5	0	3.3	4.3	3.9
Cephalothin	0.7	0	0	6.5	1.8
Lincomycin	8.3	25	10.0	28.3	12.7
Erythromycin	21.4	0	30.0	36.9	25.0
Tetracycline	57.9	0	46.7	56.5	54.8
Ampicillin	78.6	25	53.3	54.4	69.3
Penicillin G	86.9	100	66.7	73.9	81.6
Methicillin	8.9	0	13.3	26.1	13.2

[a] Number in parentheses indicates number of cultures in biotype.
[b] Includes three cultures unclassified as to biotype.

contain *S. epidermidis*. Because of the difficulty in determining the role of this organism as an etiological agent, the laboratory and clinical data of 80 patients selected at random were analyzed. Three states of clinical significance were established: urinary tract infection (UTI), indeterminate UTI, and negative UTI. Three findings were required for a UTI: (i) symptomology, including local pain, dysuria, or frequency; (ii) pyuria consisting of >10 leukocytes/high-power field; and (iii) either the presence in urine of *S. epidermidis* in pure culture in numbers $>10^5$/ml or in mixed culture in two or more specimens in numbers $>10^4$/ml. Indeterminate UTI consisted of the presence of any two of the above three findings, whereas negative UTI consisted of the presence of either one or none of the above findings. Organisms in biotype 1 were the most common organism for all states of clinical significance and were found in 18 (66.7%) of the 27 patients who had a UTI according to our criteria (Table 5). In all, 27 (33.8%) of the 80 urine isolates of *S. epidermidis* were associated with a UTI.

DISCUSSION

The biotyping scheme of Baird-Parker applied to cultures of *S. epidermidis* in the hospital environment has shown that organisms in biotype 1 are the most commonly found subgroup in hospitalized patients (Table 1). The large number of organisms of biotype 1 in urine is in agreement with others (14, 18), whereas we failed to find many organisms of biotype 2. The occurrence of different biotypes in all categories of clinical sources precludes the use of biotyping in epidemological studies, although biotyping has been useful in studies of the distribution of subgroups of *S. epidermidis* in nature and might be helpful in investigating a possible common source outbreak of *S. epidermidis* infections (4, 5). The bacteriophages specific for *S. epidermidis* isolated by Verhoef and co-workers (23) have not proven useful for identification of strains isolated in this country by us (unpublished data) and by others (13). Our laboratory is currently isolating phages from cultures employed in this study for use as typing phages in epidemiological studies (15).

It is well known that *S. epidermidis* produces a number of virulence factors that resemble those produced by *S. aureus* (8, 12). Although the production of coagulase is the most reliable test for pathogenicity, it is generally accepted that the ability of *S. aureus* to produce disease results from a combination of microbial virulence factors and host resistance. Since parameters for the identification of potentially pathogenic cultures of *S. epidermidis* have not been established, one aim of this study was that the production of virulence factors by cultures from hospitalized individuals would prove valuable in assessing the implication of isolation of certain biotypes from compromised hosts. Organisms in biotype 1 were the most enzymatically active (Table 2), and Baird-Parker's biotypes might serve to distinguish between the harmless saprophytes of this species and those more potentially capable of producing disease. Indeed, biotype 1 has been found more often than other biotypes in UTIs (14), endocarditis (21), colonization of artificial internal protheses (9), and in the lesions of acne (20). Although Baird-Parker has reported that certain members of biotype 1 are more potentially pathogenic than others, no tests to identify these were recommended (5).

It has been reported (18) that organisms in biotype 1 were significantly more active in the production of urease, and this is in agreement with our results (not shown). The importance of this enzyme in the pathogenesis of UTIs has been

TABLE 5. *Clinical significance of 80 bacteriuric cultures of S. epidermidis according to biotype*

Clinical significance	Pure or mixed culture	No. and % of cultures in biotype:			
		1[49][a]	2[1]	3[9]	4[21]
UTI	Pure	4	0	2	0
	Mixed	14	0	1	6
	Total	18 (36.7)[b]	0	3 (33.3)	6 (28.6)
Indeterminate UTI	Pure	6	0	0	2
	Mixed	5	0	1	4
	Total	11 (22.4)	0	1 (11.1)	6 (28.6)
Negative UTI	Pure	15	0	2	5
	Mixed	5	1	3	4
	Total	20 (40.8)	1 (100)	5 (55.6)	9 (42.9)

[a] Number in brackets indicates number of cultures in biotype.
[b] Number in parentheses indicates percentage of cultures with specified clinical significance.

considered for other bacterial species (18). Interestingly, the incidence of urease production was the lowest for the urinary isolates in our study. It also has been reported (9) that the production of lysozyme is more frequent among isolates from the urinary tract. However, the production of this enzyme was among the lowest for this group of isolates in our study (not shown). Interestingly, cultures from urine were among the least enzymatically active (Table 3). Although the ability of *S. epidermidis* to produce lipases has been implicated in the pathogenesis of acne (19), this activity was not high in cultures isolated from the skin in this investigation, although it must be stressed that cultures in our study were not from individuals with acne. That delta-hemolysin is the most common hemolysin produced by *S. epidermidis* (10) is in agreement with our findings in which 81.1% of our cultures produced this hemolysin (not shown). The percentage (76.3%) of cultures sensitive to lysostaphin in our study agrees with that obtained by others (17) and confirms that lysis by this enzyme may be useful to distinguish staphylococci from micrococci, but is not sensitive enough to be used alone.

We reported the greater resistance to antibiotics of *S. epidermidis* as compared to *S. aureus* earlier (12), and the resistance observed in this study make it imperative that coagulase-negative isolates be tested carefully in the laboratory prior to administration of an antibiotic. Although some individual differences in resistance were observed with the different biotypes, no consistent pattern was apparent (Table 4). The rates of resistance of *S. epidermidis* to ampicillin and penicillin observed in our laboratory are somewhat higher than those reported to exist nationally at the time the cultures were collected (2); such discrepancies may conceivably be due to geographical variation, as suggested by Pulverer et al. (16).

The results in Table 5 show that organisms in biotype 1 were most commonly associated with UTI. The incidence of the different biotypes in urine (Table 1) is similar to the incidence of different biotypes causing UTI (Table 5). It can be seen that one-third of the 80 bacteriuric cultures of *S. epidermidis* selected at random were associated with a UTI. In view of this, the presence of this organism in the approximately 700 urine cultures collected during the period of this investigation takes on added significance. Using the technique of suprapubic bladder aspiration, Bailey reported recently (3) that *S. epidermidis* was a frequent urinary tract pathogen in sexually active woman and that its presence in urine should not always be considered as an insignificant contaminant. The ubiquitous nature of this organism and the presence of certain biotypes more active in the production of virulence factors warrant increased consideration of *S. epidermidis* as an opportunistic pathogen, especially among compromised hosts in a hospital environment.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the University of Missouri-Columbia Research Council.

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