Efficacy of Phage Typing Epidemiologically Related *Staphylococcus epidermidis* Strains

LOUIS E. BLOUSE,* LAURENCE N. KOLONEL,¹ CHARLES A. WATKINS, AND JOHN M. ATHERTON

Disease Surveillance Branch, Epidemiology Division, U.S. Air Force School of Aerospace Medicine,
Brooks Air Force Base, Texas 78235

Received for publication 18 June 1975

A total of 118 epidemiologically related *Staphylococcus epidermidis* strains from hospital patients, staff, and fomites were examined with a provisional set of 18 typing phages. Seventy (59.3%) of these strains were typed using phage concentrations of 130 times routine test dilution. The remainder were nontypable. Thirty-six (30.5%) of the strains were of related phage types, 71/108/275A/459 and 71/108/275A. These latter strains were associated with clinical *S. epidermidis* endocarditis in patients with prosthetic valve replacements. Ninety-eight strains were characterized by the Baird-Parker biotyping schema. Eighty-three (84.7%) were biotype 1, and the majority (68.4%) of these were resistant to penicillin, ampicillin, methicillin, cephalothin, erythromycin, and clindamycin. Type 71, 71/108/275A/459, 71/108/275A, and 71/108/275A/459 strains were generally resistant to penicillin, ampicillin, erythromycin, and methicillin, whereas a less consistent resistance pattern was noted among miscellaneous and nontypable strains.

In the summer of 1973, an increased incidence of *Staphylococcus epidermidis* (coagulase-negative staphylococcus) infections was noted at a large stateside Air Force hospital. The majority of these infections were clustered among patients in the thoracic surgery service who had recently undergone cardiovascular surgery. Many of the strains isolated from patients and hospital staff associated with this service showed extensive patterns of antimicrobial resistance, suggesting that the strains were of hospital origin. To provide more complete laboratory information for an epidemiologic investigation of this problem, a recently described provisional set of typing phages (9) was used in typing these strains. The following is an account of the efficacy of these new typing phages in differentiating a number of epidemiologically related strains. Other methods of distinguishing these strains, including biotyping and antimicrobial resistance patterns, are presented for comparative assessment.

MATERIALS AND METHODS

Phages and host strains. Eighteen *S. epidermidis* typing phages and their respective bacterial host strains were kindly supplied by C. P. A. van Boven, Erasmus University, Rotterdam, Netherlands. This phage set included the following phage/host combinations: 40/81, 51/41, 71/240, 71A/382, 82/87, 82A/368,

108/407, 130/87, 130A/141, 157/382, 275/98, 275A/380, 275B/48, 448/407, 456/76, 459/407, 471/330, and 489/407. The source and properties of these phages have been described previously by Verhoef et al. (9, 10).

Phage propagation and typing. Standard phage typing procedures and propagation techniques (3) were used throughout this study. Trypticase soy agar and Trypticase soy broth supplemented with 400 μ g of CaCl_2 per ml were used for propagation and subsequent phage typing. Initially, strain isolates were phage typed at routine test dilution (RTD) and 100 times RTD; however, this dual typing procedure was discontinued later and only reactions of 100 times RTD were recorded as suggested by van Boven (personal communication).

Staphylococcal strains. A total of 118 *S. epidermidis* strains from patients, hospital staff, and fomites was examined. The majority were from the thoracic surgery service, although strains were also collected from other services, e.g., infectious disease, nursery, pediatrics, and orthopedics. Fourteen strains isolated from clinical services at another Air Force hospital located overseas were included for comparison.

Identification of staphylococcal strains. Suspected staphylococcal isolates were identified at the respective hospitals on the basis of typical clonal and Gram stain morphology, and by the production of coagulase and catalase. The fermentation of glucose and mannitol, the reduction of nitrate, and the production of acetylmethyl carbinol (Voges-Proskauer test) were used to separate *S. epidermidis* from *Planococcus* sp., *Aerococcus* sp., and *Micrococcus* sp. A subsample of *S. epidermidis* strains was

¹ Present address: School of Public Health, University of Hawaii, Honolulu, Hawaii 96882.

further biotyped (types 1 through 4) with a modification of Baird-Parker schema with aerobic metabolism of lactose, maltose, and mannitol (1).

Antimicrobial susceptibility tests. Strains were tested for antimicrobial susceptibility by the Bauer-Kirby method (2) with ampicillin (10 µg), cephalothin (30 µg), clindamycin (2 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamycin (10 µg), methicillin (5 µg), penicillin-G (10 U), and tetracycline (30 µg) disks. Twenty-six strains from the patients and staff on the thoracic surgery ward and service were selected for further susceptibility testing using 11 additional antimicrobials, i.e., aureomycin (30 µg), bacitracin (10 U), carbenicillin (50 µg), gantrisin (2 µg), kanamycin (30 µg), lincomycin (2 µg), minocycline (30 µg), neomycin (30 µg), novobiocin (30 µg), streptomycin (10 µg), and vancomycin (30 µg).

RESULTS

Identification and biotyping of *S. epidermidis* strains from hospital patients, staff, and fomites. A total of 131 specimens from the stateside hospital were presumptively identified as *S. epidermidis* based on the routinely used diagnostic criteria of clonal and microscopic morphology, and catalase and coagulase production. However, on further examination, 13 (10%) of these strains were found to be either *Micrococcus* sp. or *Aerococcus* sp. Of the 118 confirmed *S. epidermidis* strains, 48 (41%) were from the patients or staff on the thoracic surgery service while the remaining isolates included specimens from patients, staff, and fomites in general surgery, nursery/pediatrics, infectious disease, and the operating and recovery rooms. Two (12.5%) of the 16 presumptively identified *S. epidermidis* cultures from the overseas hospital were found on further examination to be *Micrococcus* sp.

Ninety-eight of 132 *S. epidermidis* strains were biotyped and the majority, 83 (84.7%), of

these hospital strains (patients, staff, or fomites) belonged to biotype 1. Biotype 1 strains have been shown to predominate in hospital environments (7, 8). Only four (4.1%) of the strains were biotype 3, and five (5.1%) were biotype 4. All biotype 4 strains were recovered from the overseas hospital patients, whereas only biotype 1 and 3 strains were recovered from patients at the stateside facility.

Antimicrobial susceptibility and phage typing of *S. epidermidis* strains. The antimicrobial susceptibility of the various *S. epidermidis* biotypes is shown in Table 1. The biotype 1 strains were usually resistant to ampicillin, erythromycin, clindamycin, methicillin, cephalothin, and penicillin, with 68.4% of the strains exhibiting a common resistance pattern. This same resistance pattern was also noted with the other two biotypes. Because of the highly resistant nature of the strains (Table 1), a subsample of epidemiologically significant *S. epidermidis* isolates from the thoracic surgery staff and patients was selected for more exhaustive antimicrobial testing (Table 2). Of the 26 strains tested, 22 (84.6%) showed a similarity in resistance to six of these additional antimicrobials (carbenicillin, gantrisin, kanamycin, lincomycin, neomycin, and streptomycin). Again, overlapping resistance patterns prevented further strain differentiation.

A correlation of the antimicrobial resistance and phage types is shown in Table 3. The type 71, 71/108/275A/459, 71/108/275A, and 71/108/275/459 strains were uniformly resistant to penicillin and ampicillin and were often resistant to erythromycin and methicillin as well. A similar but less consistent pattern of resistance was noted among the miscellaneous and nontypable strains examined.

During the initial phage typing attempts,

TABLE 1. Antimicrobial susceptibility testing of 98 *Staphylococcus epidermidis* strains isolated from clinical sources

Antimicrobial agent	No. (% resistant)				Totals
	Biotype				
	1	3	4	Undetermined	
Penicillin	81(97.6%)	4(100)%	4(80%)	6(100%)	95(96.9%) ^a
Ampicillin	69(83.1%)	4(100%)	4(80%)	3(50%)	80(81.6%)
Erythromycin	72(86.7%)	3(75%)		5(83.3%)	80(81.6%)
Methicillin	67(80.7%)	2(50%)		5(83.3%)	74(75.5%)
Clindamycin	62(74.7%)	3(75%)		4(66.7%)	69(70.4%)
Cephalothin	60(72.3%)	2(50%)		5(83.3%)	67(68.4%)
Tetracycline	38(45.8%)	2(50%)	3(60%)	2(33.3%)	45(45.9%)
Chloramphenicol	23(27.7%)			2(33.3%)	25(25.5%)

^a Three of the 98 *S. epidermidis* strains were sensitive to penicillin.

reactions at RTD were considered generally unsatisfactory because of their overall weak lytic

TABLE 2. Antimicrobial susceptibility testing of 26 selected *Staphylococcus epidermidis* strains from the thoracic surgery patients and staff

Antimicrobial agent	<i>S. epidermidis</i> strains showing resistance ^a	
	No.	% of total
Carbenicillin	26	100.0
Gantrisin	26	100.0
Lincomycin	25	96.2
Neomycin	23	88.5
Streptomycin	23	88.5
Kanamycin	22	84.6
Minocycline	4	15.4
Bacitracin	0	0
Novobiocin	0	0
Gentamycin	0	0
Vancomycin	0	0

^a Strains showing an intermediate sensitivity reaction were included among those strains classed as resistant.

appearance even though the typing phages were titred within 24 h of the test. These reactions resembled inhibition reactions with a faint turbid appearance within the typing drop area. Only one phage (type 71) gave clearly discernible lytic reactions at RTD. By increasing the phage concentrations to 100 times RTD, the lytic reactions were distinguished easily.

Phage typing results for 118 *S. epidermidis* isolates from clinical sources are shown in Table 4. Seventy (59.3%) of these strains were typable using the 18 provisional typing phages at 100 times RTD and were classed as biotype 1 or 3. Overall, 36 (30.5%) of the 118 strains tested were of phage type 71/108/275A/459 or of a related type 71/108/275A. These strains were shown by epidemiologic investigation (to be reported elsewhere) to be strongly associated with approximately 57.1% of the clinical *S. epidermidis* nosocomial infections. It is interesting to note that subsequent to the *S. epidermidis* outbreak, a limited number of strains were phage typed as a part of a continuing hospital surveillance program for *S. epidermidis*. The over-

TABLE 3. Antimicrobial resistance and phage types of 98 *Staphylococcus epidermidis* strains

Phage type	No. tested	Antimicrobial resistance ^a to:								
		P	AM	E	DP	CC	CF	Te	C	GM
71	13	13	13	12	10	12	0	6	7	0
71/108/275A/459	34	34	34	30	32	30	2	13	4	0
71/108/275A	2	2	2	1	1	1	1	2	1	0
71/108/275/459	2	2	2	2	2	1	0	1	0	0
Miscellaneous ^b	14	14	13	11	12	11	10	5	7	0
Nontypable ^c	33	33	27	30	21	23	25	18	11	1

^a P, Penicillin; AM, ampicillin; E, erythromycin; DP, methicillin; CC, clindamycin; CF, cephalothin; Te, tetracycline; C, chloramphenicol; GM, gentamicin.

^b Miscellaneous-unrelated phage types which included 40, 157, 275, 71/108, 51/71/108, 71/108/456/459, 71/82A/108/456, 71/275A/459, 71/82A/108/275A/456/459, 71/71A/108/459, 108/275/459, 71/82A/108/456/459/489, 71/108/275A/456/459/489, 71/108/275A/459/489, 71/71A/108/275A/459, 51/71/108/157/459/489, 40/82A/108/130A/459/471/489, 71/82/108/130/275A/275B.

^c Nontypable strains refractile to typing phages at 100 times RTD.

TABLE 4. Distribution of phage types among 118 *Staphylococcus epidermidis* isolates from specified clinical sources

Phage type	Source of strains					No.	%
	Blood	Wound	Spinal fluid	Nose	Fomite		
71	1	2		4	7	14	11.9
71/108/275A/459	5	5		11	13	34	28.8
71/108/275A	1			1		2	1.7
71/108/275/459	2					2	1.7
Miscellaneous ^a	6	3		3	6	18	15.3
Nontypable ^b	12	10	1	11	14	48	40.7

^a Miscellaneous-unrelated phage types which included 40, 157, 275, 71/108, 51/71/108, 71/108/456/459, 71/82A/108/456, 71/275A/459, 71/82A/108/275A/456/459, 71/71A/108/459, 108/275/459, 71/82A/108/456/459/489, 71/108/275A/456/459/489, 71/108/275A/459/489, 71/71A/108/275A/459, 51/71/108/157/459/489, 40/82A/108/130A/459/471/489, and 71/82/108/130/275A/275B.

^b Nontypable strains refractile to typing phages at 100 times RTD.

whelming majority (76.5%) of these strains were nontypable, and those that did type were of phage types apparently unrelated to strains previously associated with hospital infections.

DISCUSSION

A number of members of the *Micrococcaceae* have been associated with nosocomial infections. One member, *S. epidermidis*, has recently gained attention as an "emerging pathogen," particularly in severe and fatal infections in patients undergoing valvular surgery (8). Although the significance of *S. epidermidis* isolated from the blood stream has often been overlooked, the routine laboratory identification of this microorganism has never presented a problem. In fact, this study has shown that routine protocols are fully capable of identifying these strains. Even using more elaborate protocols, only a small proportion (about 10%) of the strains was identified as other members of the *Micrococcaceae*, i.e., *Micrococcus* sp. and *Aerococcus* sp. It is significant that two isolates which by earlier taxonomic criteria (4) would be identified as *Gaffkya tetragena* were typed and found susceptible to the *S. epidermidis* phages, a finding which supports the contention that *S. epidermidis* and *G. tetragena* are indistinguishable (5).

The majority of *S. epidermidis* strains which predominate in hospital environments and for which a pathogenetic role has been suggested belong to biotype 1 (6). This same singularity of biotype was documented among strains associated with the stateside hospital environment, and biotyping per se offered little supportive information for strain characterization.

The use of antimicrobial resistance patterns also failed to clearly differentiate among the various hospital strains. In fact, considerable overlapping of resistance patterns was found to exist between strains of biotype 1, 3, and 4. This overlapping persisted even though additional antimicrobials were tested. Subsequent correlation of antimicrobial resistance and phage type showed a similar overall trend of broad resistance among the various typable and nontypable strains.

Biotype 1 strains have been commonly associated with man as skin contaminants and, as such, they are frequently found in infected lesions. To evaluate the clinical significance of these strains, a new provisional phage typing set was developed (8, 9). With this set, a significant number of hospital *S. epidermidis* strains were typed and classified into five major groups, including a miscellaneous and a nontypable group. Dean and her co-workers have re-

ported similar success in phage typing biotype 1 strains with a modified set of 27 typing phages (6).

Strain 71/108/275A/459 or closely related strains (i.e., those showing the loss of a single phage typing reaction) were associated frequently with bacterial endocarditis in patients with prosthetic valve replacements and with staff personnel on the thoracic surgery service. A similar strain (71/108/275A) was found by Dean (personal communication) to predominate in this same category of patient. It should be noted that typing phage 459 was not used by Dean because of its redundancy with typing phage 108.

The problems of phage instability and the interpretation of phage types are inherent with any new phage typing system. Difficulties in each of these areas were encountered in the present study; however, the usefulness of the provisional phage typing set in differentiating a number of epidemiologically related strains has been clearly shown. The continued use of these and other new typing phages should prove valuable in monitoring the present and future impact of nosocomial disease due to *S. epidermidis*.

ACKNOWLEDGMENTS

The research reported in this paper was conducted by personnel of the Epidemiology Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks AFB, Texas.

LITERATURE CITED

1. Baird-Parker, A. C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. *J. Gen. Microbiol.* 30:409-427.
2. Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turk. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 45:493-496.
3. Blair, J. E., and R. E. O. Williams. 1961. Phage typing of staphylococci. *Bull. W. H. O.* 24:771-784.
4. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. *Bergey's manual of determinative bacteriology*, p. 466-467. Williams and Wilkins Co., Baltimore.
5. Buchanan, R. E., and N. E. Gibbons. 1974. *Bergey's manual of determinative bacteriology*, p. 478. Williams and Wilkins Co., Baltimore.
6. Dean, B. S., R. E. O. Williams, F. Hall, and J. Corse. 1973. Phage typing of coagulase-negative staphylococci and micrococci. *J. Hyg.* 71:261-270.
7. Holt, R. J. 1969. The classification of staphylococci from colonized ventriculo-atrial shunts. *J. Clin. Pathol.* 22:475-482.
8. Parisi, J. T. 1973. *Staphylococcus epidermidis*, an emerging pathogen. *Mo. Med.* 70:243-244.
9. Verhoef, J., C. P. A. van Boven, and K. C. Winkler. 1971. Characters of phages from coagulase-negative staphylococci. *J. Med. Microbiol.* 4:413-424.
10. Verhoef, J., C. P. A. van Boven, and K. C. Winkler. 1971. Lysogeny in coagulase-negative staphylococci. *J. Med. Microbiol.* 4:405-412.