JOURNAL OF BACTERIOLOGY, Apr., 1965
Vol. 89, No. 4

Printed in U.S.A.

Drug Resistance of Staphylococci

I. Transduction of Tetracycline Resistance with Phage Lysates Obtained from Multiply Resistant Staphylococci

SUSUMU MITSUHASHI, HIROSHI OSHIMA, UMEKO KAWAHARADA,[1] AND HAJIME HASHIMOTO

Department of Microbiology, School of Medicine, Gunma University, Maebashi, Japan

Received for publication 5 November 1964

ABSTRACT

MITSUHASHI, SUSUMU (Gunma University, Maebashi, Japan), HIROSHI OSHIMA, UMEKO KAWAHARADA, AND HAJIME HASHIMOTO. Drug resistance of staphylococci. I. Transduction of tetracycline resistance with phage lysates obtained from multiply resistant staphylococci. J. Bacteriol. 89:967-976. 1965.—Tetracycline resistance was found to be transduced with phage lysates obtained from multiply resistant strains of *Staphylococcus aureus* of human origin. With various combinations of multiply resistant donors and tetracycline (TC)-sensitive recipients, almost all of the strains were found to be competent donors. A greater percentage of group 1 staphylococci were competent recipients. Most of the TC^+ transductants were not lysogenic for the transducing phage and were unable to transduce TC resistance with their own phage lysates obtained by ultraviolet irradiation. However, the TC^+ transductants, lysogenized with transducing phage, were capable of transducing TC resistance, and some of the lysogenizations were accompanied by changes in phage type. These results suggest that the emergence of the multiply resistant staphylococci (consistently resistant to TC) can be accounted for by transduction among various strains accompanied sometimes by changes in phage typing pattern after lysogenization, and by selection through extensive use of antibiotics and chemotherapeutic agents.

After the discovery of sulfanilamide and antibiotics, the treatment of infectious lesions caused by some pathogenic bacteria has made rapid progress. Through their extensive use, however, the invaluable antimicrobial agents have created certain difficulties of their own, i.e., the emergence of resistant strains of bacteria.

Many studies have shown that the occurrence of multiple resistance in staphylococci, shigellae, and mycobacteria has introduced serious problems. Drug-resistant streptococci and pneumococci have not been encountered often. Many of the studies have revealed the features of resistance and cross-resistance patterns of the so-called "epidemic strains" of staphylococci. The multiply resistant staphylococci, which are confined to some specific phage type, have been isolated in the greatest percentage, and the degree of resistance of these strains to certain drugs is extremely high.

This paper deals with the transduction of tetracycline (TC) resistance in the presence of phage lysates of multiply resistant staphylococci; the epidemic spread of drug resistance by staphylococcal prophage will be discussed.

[1] Present address: Department of Pathology, School of Medicine, Gunma University.

MATERIALS AND METHODS

Bacterial strains. Strains used were 113 coagulase-positive *Staphylococcus aureus*, isolated from clinical sources, and 21 propagating strains of typing phages of the International Typing Series. Fifteen strains of *S. aureus* (Table 1), resistant to TC, streptomycin (SM), penicillin (PC), and sulfanilamide (SA), were selected at random from the stock culture of the present authors and used as the donor of TC resistance.

Media. Nutrient broth and nutrient agar media were used routinely for the propagation of bacteria. The nutrient broth medium consisted of 1% beef extract, 1% peptone, and 0.3% NaCl; the *p*H was adjusted to 7.2. Nutrient broth plus 1.5% agar was used as the solid medium. Heart Infusion (HI) agar (Difco) was used for the preparation of phage lysates from the donor strains and for the selective medium in transduction. The HI plate was also used for the determination of phage type and of drug resistance. Staphylococcal strains used were stocked in cooked meat medium (Difco). Mueller Hinton agar (Difco) was used for the determination of SA resistance.

Determination of drug resistance. All bacterial suspensions were prepared in nutrient broth and grown in stationary culture for 18 hr at 37 C. Each culture was spotted onto HI agar plates, containing serial twofold dilutions of each drug. Drug resistance was expressed as the maximal concen-

tration of each drug which allowed the growth of bacteria.

Phage typing. Phage typing was carried out according to the method described by Blair and Williams (1961). The bacteriophages and their propagating strains of the International Typing Series were received from the *Staphylococcus* Reference Laboratory, Colindale, London, England, through K. Ishihara, a member of the subcommittee in Japan.

Preparation of phage lysate from the donor strains. All bacterial suspensions were prepared in nutrient broth, grown in stationary culture for 18 hr, and diluted 100-fold with fresh nutrient broth. A 10-ml amount of a freshly prepared bacterial suspension was aerated on a shaking machine for 4 hr; about 10^9 bacterial cells per milliliter were obtained. The cells were harvested from the broth culture by centrifugation. The sedimented cells were suspended in 9 ml of saline and irradiated in an open petri dish (9 cm in diameter) placed 30 cm away from a 15-w germicidal lamp and exposed for 30 sec while shaken gently by hand. After ultraviolet irradiation, 1 ml of the 10-fold concentrated nutrient broth was added to a petri dish, which was then incubated at 37 C on a shaking machine. After incubation for 4 hr, the culture was centrifuged, and the phage lysate was obtained from the supernatant liquid by filtering through an HA Millipore disc.

Plaque formation of each lysate was checked on a HI agar plate seeded with each of the propagating strains of the International Typing Phages and of the stock culture strains of *S. aureus* isolated by the present authors. The determination of plaque-forming titers (PFT) of phage lysates was carried out by the agar-layer technique as described by Swanstrom and Adams (1951), with the use of the strain of *S. aureus* from which the highest (PFT) was obtained. A lysate from *S. aureus* MS27 showed the highest (PFT) when *S. aureus* MS353, a strain from our stock culture, was used as the indicator strain. This strain was selected from more than 100 of the stock cultures and 21 propagating strains.

Transduction. The strains of *S. aureus* isolated from clinical sources were used as the recipients of TC resistance. An overnight culture of the recipient organisms in HI broth was diluted 100 times with fresh HI broth and aerated on a shaking machine at 37 C. After 4 hr of incubation, 0.5 ml of the recipient culture was mixed with an equal volume of phage lysate of the donor strain. After 60 min of incubation at 37 C, 0.1 ml of an appropriately diluted mixture was spread onto a HI agar plate containing TC (25 μg/ml). After incubation for 48 hr, the colonies developing on the selective plate were touched with a straight needle and subjected to two successive single-colony isolations. Their drug resistance and phage type were then determined. As controls in the transduction experiment, sterility tests of the phage lysate and mutation tests of the recipient organisms were conducted in every experiment in the absence of the transducing phage.

Lysogenization. A phage lysate was obtained by ultraviolet irradiation of a strain of staphylococcus which was considered to be lysogenized. This lysate was spotted on the surface of agar plates which were previously spread with the parent strain of staphylococcus. The plates were incubated for 20 hr at 30 C. A strain was assumed to have been lysogenized when (i) the strain showed an altered phage pattern, and (ii) the phage lysate lysed the parent strain.

RESULTS

Transduction of TC resistance with phage lysates obtained from TC-resistant staphylococci. Phage lysates were obtained from 15 strains of TC-resistant staphylococci by ultraviolet irradiation as shown in Table 1. Seventeen strains of TC-sensitive *S. aureus* of various phage types and of various drug-resistance patterns were used as the recipients of TC resistance. In various combinations of donors and recipients, every strain was found to be a competent recipient of TC resistance except for *S. aureus* MS224; *S. aureus* MS27 was the most competent donor (Table 2).

The transduction of TC resistance was carried out with 94 of the TC-sensitive strains, by use of a phage lysate obtained from *S. aureus* MS27 (Table 3). Of the recipients, 48% were competent. The percentages of competent recipients found among the strains resistant to (SM.SA.PC), (SA.PC), and (SA) were 26, 52, and 48, respectively. The percentages of competent recipients found among the strains of group I, including 80/81, group II, and group O (nontypable) were

TABLE 1. *Donor strains of tetracycline resistance*

Strain	Phage-type	Drug-resistance*			
		TC	SM	SA	PC
MS27	80/81	100	100	800	200
MS31	80/81	100	100	800	200
MS47	80/81	100	100	800	200
MS58	80/81	100	100	800	200
MS60	52/52A	100	100	800	100
MS70	Nontypable	200	100	800	400
MS88	80/81	100	800	800	100
MS96	52/52A	100	100	800	100
MS97	80/81	100	100	800	25
MS129	52/52A/80/81	100	100	800	100
MS142	80/81	100	100	800	100
MS145	Nontypable	100	25	800	100
MS146	Nontypable	200	100	800	100
MS148	80/81	100	25	800	100
MS160	80/81	200	100	800	100

* Maximal concentration of the drug (micrograms per milliliter) which allows bacterial growth.

TABLE 2. *Transduction of TC resistance with phage lysates from TC-resistant staphylococci*[a]

Recipient		No. of transductants															
Strain	Phage type	Phage lysate obtained from *Staphylococcus aureus* MS															Total
		27	31	47	58	60	70	88	96	97	129	142	145	146	148	160	
MS106	80/81	12	0	6[b]	0	0	0	1[b]	13	3	0	1[b]	4	3[b]	0	0	43
MS204	29/52A/80/81	19	0	0	2[c,d]	0	0	0	0	0	0	0	0	0	3[c]	21	45
MS353	52/52A/80/81	139	0	0	0	0	2	0	0	0	0	0	2	0	0	0	143
MS362	52/52A	67	0	0	0	0	0	0	0	0	0	0	1	0	10	0	78
MS224	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MS227	71	0	0	0	0	0	0	0	0	0	0	0	0	4[b]	0	0	4
MS241	71	0	3[b]	0	0	0	0	0	0	0	1[b]	4[b]	26[b]	0	0	0	34
MS327	55/71	0	0	0	1[c]	0	0	0	0	0	0	1[b,c]	0	0	0	0	2
MS198	83A	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
S 203	7/47/53/54/75/77	0	0	(−)	0	2[b]	0	0	0	0	5[b]	0	2[b,c]	0	(−)	0	9
S 218	53/77/83A	0	0	(−)	0	0	0	0	0	0	0	0	0	1[b,c]	(−)	0	1
S 349	7/42E/83A	1	0	(−)	0	1[b,c]	0	0	0	0	0	0	0	0	(−)	0	2
S 390	7/47/53/54/75/77	6	0	(−)	0	0	0	0	0	0	0	0	0	2[b,c]	(−)	0	8
MS216	Nontypable	0	0	0	0	0	0	0	0	7[c]	0	0	0	0	0	0	7
MS320	Nontypable	34	0	0	0	0	0	0	0	0	0	0	0	0	0	1	35
MS352	Nontypable	0	0	0	9	0	0	1[c]	0	0	1[c]	0	0	0	0	0	11
MS507	Nontypable	5	0	1	0	5	0	0	0	0	0	0	0	0	0	0	11
	Total	283	3	7	12	8	2	2	13	10	7	6	35	11	13	22	434

[a] Phage lysates were obtained from the donor strains, listed in the table, by ultraviolet irradiation. Figures in the table indicate the number of colonies which developed on the selective plate containing 25 μg of TC per ml of input phage lysate. The drug resistance and phage type of the transductants were determined after two successive single-colony isolations; (−) = not done.

[b] Change of phage type to nontypable.

[c] SM resistance was transduced jointly with TC resistance.

[d] Partial change of phage type.

79, 22, and 40, respectively. *S. aureus* MS353 SA^r and *S. aureus* MS42 (SA.PC)^r were the most competent recipients among the 94 strains tested.

Relation between the time of ultraviolet irradiation for phage induction and number of transductants. A phage lysate was obtained from *S. aureus* MS27 after several ultraviolet irradiations; *S. aureus* MS353 SA^r 52/52A/80/81, *S. aureus* MS48 (SA.PC)^r 29/52/52A/70, and *S. aureus* MS42 (SM.SA.PC)^r 52/52A were used as the recipients of TC resistance (Fig. 1).

The PFT of the lysate from MS27 reached more than 10^8 per milliliter after 15 to 45 sec of ultraviolet irradiation. As can be seen in Fig. 1, the number of transductants resulting from each recipient strain parallels the PFT of the input phage lysate. Therefore, 30 sec of irradiation was adopted for use in the following experiments.

Linear relationship between the number of TC$^+$ transductants and multiplicities of infection. A phage lysate was prepared from *S. aureus* MS27 (TC.SM.SA.PC)^r 80/81 by ultraviolet irradiation. A 1-ml amount of a serially diluted phage lysate (7×10^8 per milliliter) was added to 1 ml of a recipient culture of *S. aureus* MS42 (1.3×10^9 per milliliter). Each mixture was incubated at 37 C for 60 min, chilled in ice water, and then diluted 10-fold in saline. Appropriately prepared dilutions were spread on a selective plate medium containing TC (25 μg/ml). After 48 hr of incubation at 37 C, the developed colonies were counted (Fig. 2). A linear relationship between the number of TC$^+$ transductants and multiplicities of infection was observed, indicating that the transduction of TC resistance was carried out by infection with a single transducing phage particle.

Comparison of transduction frequencies of TC resistance with recipients of various phage types. The transduction frequencies of TC resistance with a phage lysate from *S. aureus* MS27 to recipient strains of various phage types were compared. A 1-ml amount of phage lysate (1.2×10^9) and a similar amount of recipient culture were mixed and incubated at 37 C. After 30 min of incubation, the mixture was chilled in ice-cold water and centrifuged, and the PFT of the supernatant liquid was determined. There

TABLE 3. *Transduction of TC resistance from Staphylococcus aureus MS27 to the various recipient strains**

Recipient		No. of tested strains	No. of competent recipients
Drug-resistance	Phage type		
SM.SA.PC	Group I and 80/81	7	4 (52)
	Group II	5	1 (20)
	Others	0	0 (0)
	Nontypable	7	0 (0)
	Total	19	5 (26)
SA.PC	Group I and 80/81	13	11 (85)
	Group II	19	4 (21)
	Others	1	1
	Nontypable	13	8 (62)
	Total	46	24 (52)
SA	Group I and 80/81	9	8 (89)
	Group II	12	3 (25)
	Others	1	0
	Nontypable	7	3 (43)
	Total	29	14 (48)
Total		94	43 (48)

* A phage lysate was prepared from *S. aureus* MS27 according to the method described in Materials and Methods; the plaque-forming titer of its lysate was 8.5×10^8/ml when *S. aureus* MS353 was used as an indicator strain. Numbers in parentheses indicate percentage of competent recipients per total number of tested recipients.

was no significant difference in adsorption rate of phage to the recipient bacteria (Table 4). However, the transduction frequency varied markedly, and no transductants were obtained from the strains of group 11, i.e., three recipients of phage type 71 and one recipient of phage type 55/71.

Joint transduction of TC and SM resistance. As shown in Table 2, SM resistance was concomitantly transduced with TC resistance when transductants were selected on TC plates. The joint transduction of TC and SM resistance was further examined when selected either on TC or on SM plates. A phage lysate was obtained by ultraviolet irradiation of *S. aureus* MS27 (TC.SM.SA.PC)^r 80/81. *S. aureus* SA^r 52/52A/80/81 was used as the recipient. When selected on TC (25 μg/ml) plates, a 2% joint transduction was observed among 153 strains of TC$^+$ transductants. The degree of joint transduction with SM selection was about sevenfold higher than with TC selection (Table 5). The occurrence of joint transduction of TC and SM resistance, even at the multiplicities of infection of 10^{-2}, suggests the closeness in location of the genetic loci governing both TC and SM resistance.

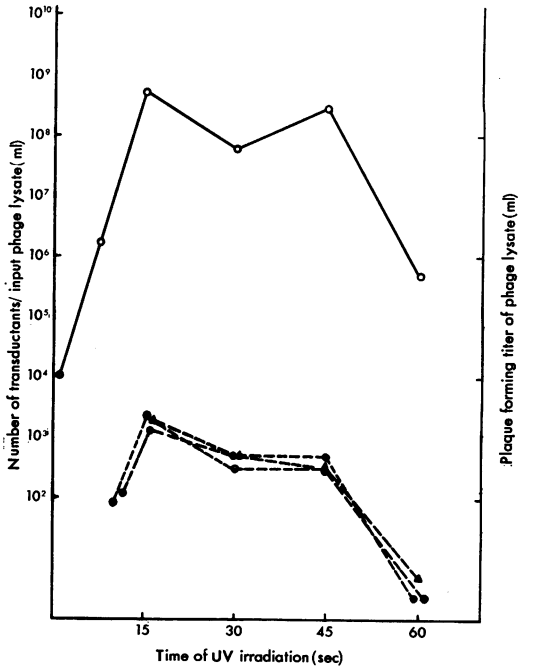

FIG. 1. *Relationship between the time of ultraviolet irradiation for phage induction and number of transductants. The phage lysate was obtained from Staphylococcus aureus MS27 by the method described in Materials and Methods. S. aureus MS353, MS48, and MS42 were used as the recipients of TC resistance, and the numbers of bacterial cells (per milliliter) were 2.4×10^9, 2.4×10^9, and 1.8×10^9, respectively. Symbols: ○, plaque-forming titer per milliliter; dotted lines indicate the number of transductants per 1 ml of input phage lysate; ◒, S. aureus MS353; ●, S. aureus MS48; △, S. aureus MS42.*

Change of phage pattern after transduction. Most of the transductants showed phage type patterns identical to those of the parent recipient strains (Table 2). However, a variety of changes was observed in the resulting phage patterns after transduction, e.g., a change to group O (nontypable) and a partial alteration from the parent strain. Three strains of *S. aureus*, MS353 52/52A/80/81, MS48 29/52/52A/79, and MS42 52/52A, were used as the recipients of TC resistance, and the change of their phage patterns after transduction was examined. *S. aureus* MS27 (TC.SM.SA.PC)^r 80/81 was used as a donor of TC resistance (Table 6). When *S. aureus* MS353 was used as a recipient, 20% of the variants were of altered phage type, a change from 52/52A/80/81 to 80/81; and 80% of the transductants showed phage patterns identical to the parent strains even after transduction of TC resistance.

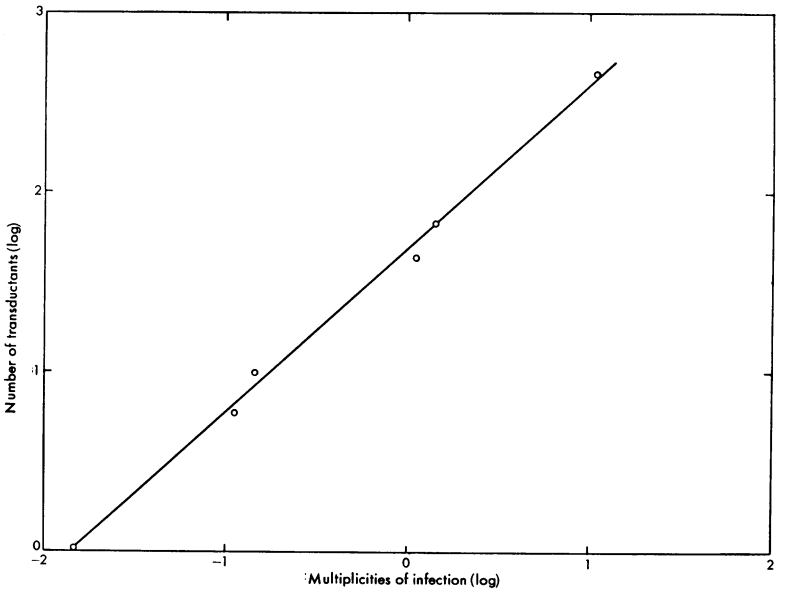

FIG. 2. *Linear relationship between the number of TC+ transductants and multiplicities of infection. A phage lysate was prepared from Staphylococcus aureus MS27 by ultraviolet irradiation. S. aureus MS353 was used as a recipient of TC resistance.*

TABLE 4. *Comparison of the transduction frequency of TC resistance with recipients of various phage types**

Recipient		Free PFT after adsorption	No. of transductants	Transduction frequency
Strain	Phage type			
MS106	80/81	3.7×10^7	96	8.2×10^{-8}
MS204	29/52A/80/81	1.5×10^6	158	1.3×10^{-7}
MS355	52/52A/80/81	8.2×10^6	828	6.9×10^{-7}
MS362	52/52A	2.2×10^7	68	5.8×10^{-8}
MS216	71	8.5×10^7	0	
MS224	71	1.0×10^6	0	
MS241	71	1.3×10^6	0	
MS327	55/71	8.0×10^5	5	4.1×10^{-9}
MS352	Nontypable	2.7×10^6	6	
MS198	83A	1.0×10^6	0	
MS320	Nontypable	1.3×10^6	42	3.5×10^{-8}
MS507	Nontypable	1.3×10^6	143	1.2×10^{-7}

* A phage lysate was prepared from *Staphylococcus aureus* MS27 by ultraviolet irradiation; the plaque-forming titer (PFT) of the input phage was 1.2×10^9. The number of bacterial cells of the recipient strains was 3×10^8 to 7×10^8 per milliliter. Transduction frequency indicates number of transductants per number of adsorbed phage particles.

No variants of phage type were obtained from MS48 and SM42 after transduction.

The TC+ transductants of phage type 80/81, obtained from *S. aureus* MS353 52/52A/80/81 with a phage lysate of MS27 80/81, were immune to the action of a phage lysate of MS27. This suggests that the TC+ transductants 80/81 were lysogenized with a transducing phage, and that they were immune not only to the transducing phage but also to typing phages 52 and 52A. The TC+ transductant, i.e., *S. aureus* MS353t 80/81, was also a competent donor of TC resistance. The phage lysate obtained by ultraviolet irradiation of MS353t 80/81 was capable of transducing TC resistance at almost the same transduction frequency as the original donor

TABLE 5. *Joint transduction of TC and SM resistance**

Selective drug	Transduction frequency	Resistance patterns of the transductants		
		TC	SM	TC.SM
TC	7×10^{-6}	150/153 (98)	0/153	3/153 (2)
SM	1.3×10^{-6}	0/153	122/153	31/153 (20)

* A phage lysate (2.0×10^7 per milliliter) was obtained from *Staphylococcus aureus* MS27. *S. aureus* MS353 (1.8×10^9 per milliliter) was used as the recipient. The numerator indicates the number of transductants resistant to the drug shown in the table, and the denominator indicates the total number of transductants tested. Numbers in parentheses show the percentage of transductants resistant to (TC.SM), i.e., joint transductants.

strain of MS27. However, transduction was not possible with the lysate obtained from MS353t 52/52A/80/81 of the same phage type as the parent recipient strain.

All TC^+ transductants obtained from the recipient strains of phage type group II or III lost their sensitivity to the standard typing phages and were converted to the nontypable strains.

Prophage typing of 15 donor strains of TC resistance. Phage lysates were obtained by ultraviolet irradiation of 15 *S. aureus* strains, i.e., the donor strains of TC resistance listed in Table 1. The lytic pattern of these phage lysates was examined by spotting one loopful of each lysate onto nutrient agar plates seeded with each propagating strain of the International Typing Phages. The propagating strains of typing phage 80 and 81 were found to be the most sensitive indicator strains, and all the donor strains used were found to be lysogenic (Table 7). The propagating strains, 52, 52A, 29, 79, 55, 71, 3A, 52, 77, 42E, 42D, and 187 did not lyse in the presence of lysates prepared from the 15 donor strains. Nontypable strains, i.e., MS145, MS146, and MS70, showed a wide spectrum in prophage typing. Thus, it will be noted that prophage typing showed a specific lytic spectrum different from the original phage type of the International Typing Series. The detailed results of staphylococcal prophage typing will be described elsewhere.

TABLE 6. *Change of the phage pattern after transduction**

Recipient		Transductant	
Strain	Phage pattern	Phage pattern	Per cent
MS353	52/52A/80/81	52/52A/80/81	148/180 (80)
		80/81	36/180 (20)
MS48	29/52/52A/79	29/52/52A/79	88/88 (100)
MS42	52/52A	52/52A	84/84 (100)

* A phage lysate (10^9 per milliliter) was obtained from *Staphylococcus aureus* MS27. *S. aureus* MS353 (2.8×10^9), MS48 (6.5×10^9), and MS42 (1.5×10^9) were used as the recipients of TC resistance. The denominator indicates the total number of colonies tested, and the numerator indicates the number of colonies of phage type shown in the table. Numbers in parentheses show the percentage of transductants of indicated phage type.

DISCUSSION

Many previous studies have been confined to determining the resistance and cross-resistance patterns of the so-called "epidemic strains" of staphylococci. The resistance patterns of 6,723 staphylococcal isolates in the United States were described by Fowler, Watters, and Levy (1963); those of 1,249 pure cultures of staphylococci obtained from various hospitals in Japan, from 1957 to 1962, were reported by the present author

TABLE 7. *Lytic pattern of the lysates obtained from 15 donor strains of TC resistance**

Lysates from		Propagating strain of typing phage										
		I	M	III						II		Others†
Strain	Phage type	80	81	47	6	7	54	75	83A	3C	3B	
MS27	80/81	+++	+++	+++	++	+	−	−	+	−	−	−
MS47	80/81	++	+++	+++	++	+	−	−	+	−	−	−
MS148	80/81	++	+++	+++	+	+	−	+	+	+	−	−
MS145	NT	++	+++	+++	++	+	+	+	+++	+	−	−
MS146	NT	++	+++	+++	++	+	−	−	+	+	−	−
MS58	80/81	++	+++	−	+	+	+	−	+	+	−	−
MS96	52/52A	++	+++	−	++	+	+	−	−	+	−	−
MS70	NT	++	+++	−	+	+	−	−	+	−	−	−
MS160	80/81	+	+	−	−	−	−	+	+	−	−	−
MS126	52/52A/80/81	+	+	−	+	−	−	−	−	−	−	−
MS60	52/52A	+	+	−	−	−	−	−	+	−	−	−
MS88	80/81	−	+	+	−	−	−	−	+	−	+	−
MS142	80/81	+	−	−	−	−	−	−	−	−	+	−
MS31	80/81	−	−	−	+	−	−	−	+	−	−	−
MS97	80/81	−	−	+	+	−	−	−	−	+	−	−

* Symbols: +++, confluent lysis; ++, near confluent lysis and uncountable plaque formation; −, no plaque formation.

† Others include the typing phages 52, 52A, 79, 29, 55, 71, 3A, 53, 77, 42E, 42D, and 187.

(Mitsuhashi, 1962). The greatest percentage of cross-resistance found was TC.SM.PC.SA (23.8%), followed by PC.SA (18.5%), TC.PC.SA (14%), and SM.PC.SA (7.7%; Mitsuhashi, 1962).

Since the appearance of the first report of an extensive staphylococcal outbreak in Australia in 1953 (Rountree, 1956), many epidemics due to staphylococci of phage type 80, occurring in maternity and surgical wards, have been recorded in Canada, Great Britain, the United States, and also in Japan (Ishihara, Tanaka, and Tajima, 1959; Mitsuhashi, 1962). These surveys disclosed that the most striking feature of the staphylococci was the predominance of multiple resistance and of specifically confined phage type. It was noted that the degree of resistance to each drug was extremely high in the staphylococcal strains with multiple resistance (Mitsuhashi, 1962; Mitsuhashi, Hashimoto, and Egawa, *in preparation*).

To explain the manifestations of multiple resistance and restricted phage-typing patterns by the epidemic *S. aureus* strains, the following possibilities are presented: (i) mutation and selection through extensive use of therapeutic agents and (ii) presence of additional factors, i.e., plasmid (or episomes) including prophage.

It is known that multiple resistance is predominant in shigellae and staphylococci, whereas drug resistance is not often encountered in strains of streptococci and pneumococci. However, the in vitro mutation rate of strains of staphylococci to drug resistance is not always higher than those of pneumococci and streptococci (Mitsuhashi, *unpublished data*). This fact suggests that mutation and selection are not solely responsible for the multiple resistance of staphylococci and for their epidemic spread.

In the study of epidemic spread of multiply resistant shigellae, it was found that multiple resistance (resistance to TC.CM.SM.SA) was transmitted between shigellae and *Escherichia coli* by mixed cultivation (Ochiai et al., 1959; Akiba et al., 1960). This transmissible drug-resistance factor is transmitted among the strains of the family Enterobacteriaceae (Harada et al., 1960) and is mediated not by transduction or transformation but by conjugation (Mitsuhashi, Harada, and Hashimoto, 1960). As a result of the successful artificial elimination of the transmissible drug-resistance factor by treatment with acriflavine (Mitsuhashi, Harada, and Kameda, 1961*b*), the term "R" (resistance) was proposed for this transmissible drug-resistance factor (Mitsuhashi, 1960). This term is analogous to the term "F" for the sex factor of *E. coli* (Lederberg, Cavalli, and Lederberg, 1952). Thus, the epidemic spread of multiple resistance by strains of shigellae is accounted for by the existence of the transmissible drug-resistance factor, R.

It was reported by the present authors that, in staphylococci, the capacity to produce penicillinase and the resistance to macrolide antibiotics, i.e., erythromycin, oleandomycin, leucomycin, and spiramycin, are controlled by a genetic element which exists extrachromosomally (Mitsuhashi et al., 1963; Hashimoto, Kono, and Mitsuhashi, 1964). Furthermore, the determinants of penicillinase production and of resistance to macrolide antibiotics are jointly transduced with a phage lysate obtained from multiply resistant staphylococci (Mitsuhashi et al., 1965). It was also independently described that the determinant of penicillinase production in staphylococci is controlled by a cytoplasmic element (Novick, 1963; Harmon and Baldwin, 1964). The fact that the characters of staphylococcal drug resistance are controlled by genetic traits located both on the chromosome and on a plasmid (or episome) should be considered in any explanation of the epidemic features of staphylococcal drug-resistance and phage-typing patterns.

It is known that almost all of the staphylococcal strains are lysogenic. Many studies concerned with staphylococcal phage typing have generally assumed that at least some of the differences between strains belonging to the same phage group but displaying different phage patterns are due to the presence in these strains of prophages which confer immunity to homologous and related phages. It has been recorded by many research workers that staphylococcal strains are altered in their phage sensitivity after artificial lysogenization (Lowbury and Hood, 1953; Rountree, 1956, 1959; Asheshov and Rippon, 1959; Sakuri et al., 1961; Blair and Carr, 1961).

Transduction in *S. aureus* was first reported by Ritz and Baldwin (1961), who demonstrated that several phages of the International Typing Series were capable of transducing the capacity to produce penicillinase among a number of these strains. Morse (1959) transduced resistance to streptomycin and novobiocin in *S. aureus* with an ultraviolet-inducible mutant of typing phage 53. It was also described that phages 80, 29, 52A, 79, and 53 of the International Typing Series were capable of transducing resistance to chlortetracycline and novobiocin and the capacity to produce penicillinase (Pattee and Baldwin, 1961).

It was demonstrated by the present authors that resistance of *S. aureus*, obtained from clinical sources, to tetracycline is transduced with phage 80 (Mitsuhashi et al., 1961*c*), and with phage 80 and 81 (Niwa et al., 1962). It was also found by the present authors (Mitsuhashi

et al., 1962, 1963, 1965) that resistance to erythromycin is transduced in staphylococci with phages 80 and 81, and that resistance to macrolide antibiotics (erythromycin, leucomycin, oleandomycin) is jointly transduced with phage lysates obtained by ultraviolet induction of the resistant strains of *S. aureus*. It was further demonstrated in this laboratory that the capacity to produce penicillinase and the ability to resist macrolide antibiotics (erythromycin, oleandomycin, leucomycin, spiramycin) are jointly eliminated by treatment of the staphylococcus with acriflavine and are jointly transduced with phage lysates obtained from multiply resistant strains of staphylococci isolated from clinical sources (Hashimoto et al., 1964; Mitsuhashi et al., 1965). Pattee and Baldwin (1962) reported that resistance to erythromycin and oleandomycin were transduced with phages 29, 52A, 78, 80, and 53 of the International Typing Series, which were employed as transducing phages.

The transduction of bacterial characters by bacteriophage particles is of two types, specialized (Morse, Lederberg, and Lederberg, 1956*a,b;* Arber, Kellenberger, and Wigle, 1957) and general (Zinder and Lederberg, 1952; Lennox, 1955; Jacob, 1955). In general transduction, the transductants with P1 in a single infection are generally sensitive to P1 (Adams and Luria, 1958). This indicates the absence or nondetectable existence of small portions of the P1 genome in the tranducing particles, although a special case of transduction, i.e., an active transducing phage P1 *CM* produced by combination with an R factor, has been reported (Kondo and Mitsuhashi, 1964). In an analogy to these facts, the functioning of the TC transducing particle, described in the present article, can be interpreted as belonging to the category of general transduction. Transduction can be accomplished by a phage grown on the donor strain by a lytic cycle, and the transductants at low multiplicity of infection are generally sensitive to the transducing phage.

It is proposed that transduction and lysogenization by phages and the selection of these strains through extensive use of therapeutic agents are responsible for the wide distribution of multiply resistant staphylococci of restricted phage-typing patterns.

ACKNOWLEDGMENTS

We wish to thank H. Umezawa and A. C. Sanders for their encouragement during the course of this study. We gratefully acknowledge the technical assistance of Mitsuko Tsunoda in conducting this work.

This investigation was supported by U.S. Army research grant DA-CRD-AG-G19 from U.S. Army Research and Development.

LITERATURE CITED

ADAMS, J. N., AND S. E. LURIA. 1958. Transduction by bacteriophage P1. Abnormal phage function of the transducing particles. Proc. Natl. Acad. Sci. U.S. **44**:590–594.

AKIBA, T., T. KOYAMA, S. ISHIKI, S. KIMURA, AND T. FUKUSHIMA. 1960. Studies on the mechanism of development of multiply drug-resistant *Shigella* strains. Nihon Iji Shimpo No. 1866, p. 45–50.

ARBER, W., G. KELLENBERGER, AND J. WEIGLE. 1957. La défectuosité du phage lambda transducteur. Z. Allgem. Pathol. Bakteriol. **20**:659–665.

ASHESHOV, E. H., AND J. E. RIPPON. 1959. Changes in typing pattern of phage-type 80 staphylococci. J. Gen. Microbiol. **20**:634–643.

BLAIR, J. E., AND M. CARR. 1961. Lysogeny in staphylococci. J. Bacteriol. **82**:984–993.

BLAIR, J. E., AND R. E. O. WILLIAMS. 1961. Phage typing of staphylococci. Bull. World Health Organ. **24**:771–784.

FOWLER, J. R., WATTERS, J. L., AND A. M. LEVY. 1963. Bacterial susceptibility patterns as related to geographic variation and anatomical source. Clin. Med. **70**:547–558.

GRAESSLE, O. E., AND J. J. PIETROWSKI. 1949. The in vitro effect of para-aminosalicylic acid (PAS) in preventing acquired resistance to streptomycin by Mycobacterium tuberculosis. J. Bacteriol. **57**:459–464.

HARADA, K., M. SUZUKI, M. KAMEDA, AND S. MITSUHASHI. 1960. Transmission of drug-resistance among *Enterobacteriaceae*. Japan. J. Exptl. Med. **30**:289–299.

HARMON, S. A., AND J. N. BALDWIN. 1964. Nature of the determinant controlling penicillinase production in *Staphylococcus aureus*. J. Bacteriol. **87**:593–597.

HASHIMOTO, H., AND S. MITSUHASHI. 1964. Studies on the drug resistance of staphylococci. J. Antibiot. (Tokyo) Ser. B. **17**:129–132.

HASHIMOTO, H., K. KONO, AND S. MITSUHASHI. 1964. Elimination of penicillin resistance of *Staphylococcus aureus* by treatment with acriflavine. J. Bacteriol. **88**:261–262.

HSIE, J., AND V. BRYSON. 1950. Genetic studies on the development of resistance to neomycin and dihydrostreptomycin in *Mycobacterium ranae*. Am. Rev. Tuberc. Pulmonary Diseases **63**:286–299.

ICHIHASHI, Y. 1964. Drug resistance pattern of pneumococci and epidemiological and genetic problems. J. Antibiot. (Tokyo) Ser. B. **17**:116–119.

ISHIHARA, K., A. TANAKA, AND S. TAJIMA. 1959. Phage-typing of staphylococci from pyogenic lesions and carriers in and outside the hospital. Japan. J. Microbiol. **3**:427–442.

JACOB, F. 1955. Transduction of lysogeny in *Escherichia coli*. Virology **1**:207–220.

KONDO, E., AND S. MITSUHASHI. 1964. Drug resistance of enteric bacteria. IV. Active transducing bacteriophage P1 *CM* produced by the combination of R factor with bacteriophage P1. J. Bacteriol. **88**:1266–1276.

KOSAKAI, N., ET AL. 1960. Studies on the drug-resistant staphylococci in Japan. Modern Med. **15**:2–8.

KOSAKAI, N., ET AL. 1961. Studies on the drug-resistant staphylococci in Japan. Modern Med. **16**:2997–3004.

LEDERBERG, J., L. L. CAVALLI, AND E. M. LEDERBERG. 1952. Sex compatibility in *E. coli*. Genetics **37**:720–730.

LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.

LOWBURY, E. J. L., AND A. M. HOOD. 1953. The acquired resistance of *Staphylococcus aureus* to bacteriophage. J. Gen. Microbiol. **9**:524–535.

MIKATA, I., AND H. TOMIOKA. 1964. Drug resistance of *Streptococcus viridans* and *Streptococcus hemolytics*. J. Antibiot. (Tokyo) Ser. B. **17**:119–126.

MITSUHASHI, S., 1960. On the genetics and drug-resistance of enteric bacteria. Kagaku (Tokyo) **30**:628–633.

MITSUHASHI, S. 1962. Drug-resistance patterns of staphylococci. Rept. Ministry of Education, Japan, p. 23–27.

MITSUHASHI, S., K. HARADA, AND H. HASHIMOTO. 1960. Multiple resistance of enteric bacteria and transmission of drug-resistance to other strain by mixed cultivation. Japan. J. Exptl. Med. **30**:179–184.

MITSUHASHI, S., K. HARADA, H. HASHIMOTO, AND R. EGAWA. 1961*a*. Drug-resistance of *Shigella* prevalent in Japan. Japan. J. Exptl. Med. **31**:47–52.

MITSUHASHI, S., K. HARADA, AND M. KAMEDA. 1961*b*. Elimination of transmissible drug-resistance factors by treatment with acriflavine. Nature **189**:947.

MITSUHASHI, S., M. MORIMURA, K. KONO, AND H. OSHIMA. 1963. Elimination of drug resistance of *Staphylococcus aureus* by treatment with acriflavine. J. Bacteriol. **86**:162–164.

MITSUHASHI, S., T. NAKANO, Y. FUKUTOMI, AND Y. KAKINUMA. 1961*c*. Transduction of drug-resistance in *Staphylococcus aureus*. Gunma J. Med. Sci. **10**:297–300.

MITSUHASHI, S., T. NAKANO, Y. FUKUTOME, AND Y. KAKINUMA. 1962. Transduction of erythromycin resistance in *Staphylococcus aureus*. Gunma J. Med. Sci. **11**:241–242.

MITSUHASHI, S., H. HASHIMOTO, M. KONO, AND M. MORIMURA. 1965. Drug resistance of staphylococci. II. Joint elimination and joint transduction of the determinants of penicillinase production and resistance to macrolide antibiotics. J. Bacteriol. **89**:988–992.

MCCORMACK, R. C., D. KAYE, AND E. W. HOOK. 1962. Resistance of group A streptococci to tetracycline. New Engl. J. Med. **267**:323–326.

MORSE, M. L. 1959. Transduction by staphylococcal bacteriophage. Genetics **45**:722–727.

MORSE, M. L., E. M. LEDERBERG, AND J. LEDERBERG. 1956*a*. Transduction in *Escherichia coli* K12. Genetics **41**:142–156.

MORSE, M. L., E. M. LEDERBERG, AND J. LEDERBERG. 1956*b*. Transduction heterogenotes in *Escherichia coli*. Genetics **41**:758–779.

NIWA, C., S. GOTO, S. KUWABARA, T. KOYAMA, AND T. AKIBA. 1963. Transduction of drug-resistance of staphylococci. Med. Biol. **66**:53–55.

NOVICK, R. P. 1963. Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus*. J. Gen. Microbiol. **33**:121–136.

OCHIAI, K., A. NAKAMURA, T. YAMANAKA, K. KIMURA, AND O. SAWADA. 1959. Studies on inheritance of drug-resistance between *Shigella* strains and *Escherichia coli* strains. Nihon Iji Shimpo No. 1861, p. 34–46.

OCHIAI, K., T. TOTANI, O. SAWADA, T. YAMANAKA, AND S. NAITO. 1960. Epidemiological study on the drug-resistant shigellae. Japan. Clin. **18**:101–106.

PATTEE, P. A., AND J. N. BALDWIN. 1961. Transduction of resistance to chlortetracycline and novobiocin in *Staphylococcus aureus*. J. Bacteriol. **82**:875–881.

PATTEE, P. A., AND J. N. BALDWIN. 1962. Transduction of resistance to macrolide antibiotics in *Staphylococcus aureus*. J. Bacteriol. **84**:1049–1055.

RITZ, H. L., AND J. N. BALDWIN. 1961. Transduction of capacity to produce staphylococcal penicillinase. Proc. Soc. Exptl. Biol. Med. **107**:678–680.

ROUNTREE, P. M. 1951. Cross-infection of surgical wounds. Med. J. Australia **2**:766–769.

ROUNTREE, P. M. 1956. Variations in a related series of staphylococcal bacteriophages. J. Gen. Microbiol. **15**:266–279.

ROUNTREE, P. M. 1959. Changes in the phage-typing patterns of *Staphylococcus* following lysogenization. J. Gen. Microbiol. **20**:620–633.

SAITO, M., ET AL. 1964. Drug-resistance pattern of resistant *Shigella* and epidemiological problems. J. Antibiot. (Tokyo) Ser. B. **17**:109–112.

SAKURI, N., E. L. UPDYKE, A. J. NAHMIAS, AND M. R. GERHARDT. 1961. Laboratory observations on the lysogenic properties of hospital staphylococci and their possible epidemiological implications. Am. J. Public Health **51**:566–576.

SWANSTROM, M., AND M. H. ADAMS. 1951. Agar layer method for production of high titer phage stocks. Proc. Soc. Exptl. Biol. Med. **78**:372–375.

SZYBALSKI, W., AND V. BRYSON. 1954. Genetic

studies on microbial cross-resistance to toxic agents. III. Cross-resistance of *Mycobacterium renae* to twenty-eight antimycobacterial agents. Am. Rev. Tuberc. Pulmonary Diseases **69**:267–279.

TSUKAMURA, M., AND T. ABO. 1958. Some genetic considerations on the isoniazid-resistance system of *Mycobacterium avium*. Amer. Rev. Tuberc. Pulmonary Diseases **77**:519–523.

WAISBREN, B. A., AND C. L. STRELITZER. 1958. A five year study of the antibiotic sensitivities and cross resistance of staphylococci in a general hospital. Antibiot. Ann. 1957–58, p. 350–364.

WILLIAMS, R. E., AND J. E. RIPPON. 1952. Bacteriophage typing of *Staphylococcus aureus*. J. Hyg. **50**:320–353.

YOSHIOKA, M. 1964. Drug resistance pattern of *Streptococcus hemolytics* and epidemiological and genetic problems. J. Antibiot. (Tokyo) Ser. B. **17**:126–129.

ZINDER, N. D., AND J. LEDERBERG. 1952. Genetic exchange in Salmonella. J. Bacteriol. **64**:679–699.