LYSOGENICITY OF METHICILLIN-RESISTANT STRAINS OF *STAPHYLOCOCCUS AUREUS*

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SUMMARY. The lysogenic status of 23 strains of methicillin-resistant *Staphylococcus aureus*, isolated at the Royal Prince Alfred Hospital, Sydney, since 1980, was studied. Twenty strains, belonging to the four predominant phage types isolated in this hospital, carried the same lysogenic phage which we have designated C. Three other phages were isolated from five strains belonging to phage type 84/85/90. The presence of phage C had little effect on the phage-typing pattern of the strains. Similarly, lysogenisation with the other three phages did not result in a significant change in phage-typing patterns. However, when strain 1489, isolated in 1969, was lysogenised with these three phages, there was a change in phage-typing pattern. Lysogenisation of this strain with phage 47T resulted in a marked loss of sensitivity to both group-I and group-III phages. The lysogenic status of these methicillin-resistant strains of *S. aureus* was compared with that of strains isolated between 1967 and 1970. There was no evidence that the strains isolated recently were either related to, or derived from, the earlier ones.

INTRODUCTION

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are being increasingly reported as causes of nosocomial infections in Australia (McDonald *et al.*, 1981; King *et al.*, 1982; Pavillard *et al.*, 1982) and in other countries (Schaeffer *et al.*, 1981; Linneman *et al.*, 1982; Archer and Mayhall, 1983; Cafferkey *et al.*, 1983). These strains are difficult to type with the Basic International Set of phages (Archer and Mayhall, 1983). Schaeffer *et al.* (1981) reported that a large percentage of methicillin-resistant strains isolated in New York City was not typable with the Basic International Set when used at the routine test dilution (RTD) or at 100RTD, but was typable only by experimental phages. Cafferkey *et al.* (1983) also reported the emergence, in 1977, of strains of MRSA untypable with the routine set of phages.

The differentiation of MRSA strains, isolated at Royal Prince Alfred Hospital (RPAH), Sydney, by phage typing with the Basic International Set of phages at 100RTD and an additional set of phages has been reported (Vickery *et al.*, 1983). Since

1980, strains belonging to four phage patterns have predominated and these differed in phage type from strains isolated between 1967 and 1970 (Rountree and Beard, 1968; Rountree and Vickery, 1973). The presence of a prophage in a strain of *S. aureus* may affect the phage-typing pattern of that strain (Blair and Carr, 1961; Rountree and Asheshov 1961; Duval-Ifrah, 1972). Four typical but different earlier MRSA strains were each shown to contain two lysogenic phages (Vickery, unpublished observation) and the question arose as to whether or not the more recent strains were lysogenically similar to, or could have been derived from, the earlier strains.

MATERIALS AND METHODS

Strains of S. aureus. All strains of MRSA were isolated at the Royal Prince Alfred Hospital, Sydney. They were slide coagulase positive and were stored on nutrient-agar slopes at room temperature. Strains 1489 and 8947 have been described (Vickery *et al.*, 1983). Five strains of each of four predominant phage types isolated since 1980 were tested for the presence of lysogenic phages. A sixth strain 10335, isolated in December 1983, was also studied. Propagating and indicator strains of the Basic International Set of phages were used to determine the host ranges of all phages.

Media and antibiotic sensitivity testing. These methods and materials have been previously described (Vickery *et al.*, 1983).

Phages. Phage typing was performed by the method of Blair and Williams (1961) with the Basic International Set of 23 phages and three experimental phages, 187, 90 and 88, issued by the International Reference Laboratory, Colindale (Asheshov and Skalova, 1975); the typing patterns are reported at 100RTD. Five additional phages isolated from MRSA (Vickery *et al.*, 1983) were also used to type the strains, and the typing patterns are reported at RTD. Lysogenic phages were obtained by induction of MRSA with UV light by the method of Gorrill and Gray (1956), and propagated on strain 1489 to obviate any possible host modification. All new phages were characterised by their host range and serological type (Rippon, 1956). Lysogenic phages were numbered according to the strain of staphylococcus from which they were derived, e.g., phage 87M was a phage of medium plaque size from strain 1987 and phage 87L was a large-plaque phage from the same strain; phage 67R was a phage with a rough edged plaque from strain 8967 and phage 47T was a tiny-plaque phage from strain 8947.

Lysogenisation. Staphylococcal strains were lysogenised by spotting undiluted phage on to a lawn of cocci. After incubation overnight at 30°C, a small quantity of the secondary bacterial growth in the area of lysis was streaked on to blood agar plates. After incubation overnight at 37°C, single colonies were picked into broth and tested for the presence of the lysogenising phage, for resistance to it, and for alteration in phage-typing pattern. The lysogenised strains were numbered in the usual way, e.g., strain 1489 (87M) indicated strain 1489 lysogenised with phage 87M.

RESULTS

Lysogenicity of MRSA strains

Phages were isolated from 21 strains of MRSA (table I). Characterisation of these phages, by determining their host range and serology, showed that an identical large-plaque phage designated C (common), was isolated from all strains. From the strains belonging to phage types 83A/85/95/90/88 and 77/83A/84/85/95/90/88 and from untypable strains, this phage only was isolated. However, from three of the five strains of phage type 84/85/90, additional phages were isolated. Strain 8967 yielded phage C and phage 67R; phage C and phage 87M were isolated from strain 1987 and

TABLE I
Lysogenicity of methicillin-resistant *S. aureus*

Typing patterns with		Strain no.	Lysogenic phages isolated
Basic International Set of phages at 100RTD	Additional phages at RTD		
83A/85/95/90/88	47T/90A	346	C
		10324	C
		10318	C
		10519	C
		10347	C
77/83A/84/85/95/90/88	47T/56A/56C/90A	6333	C
		9337	C
		9378	C
		2010	C
		10531	C
Not Typable	Not Typable	675	C
		8554	C
		8134	C
		1648	C
		4112	C
84/85/90	56B/56C/90A	8947	C, 47T
		8967	C, 67R
		9164	C
		10359	C
77/85	56A/56C	1987	C, 87M
	90A	10335	C, 47T
	56A/56C		

strain 8947 yielded phage 47T as well as phage C. No additional phages were detected from strains 9164 and 10359.

Strain 10335, of phage type 77/85:56A/56C, was the first MRSA of this phage pattern to be isolated at RPAH, although identical strains had been isolated in one other Sydney hospital. Two phages, C and 47T, were isolated from this strain.

Two other MRSA, of phage type 83A/85/95/90/88:47T/90A but sensitive to phage C, were induced by UV irradiation. No lysogenic phages were detected from either strain.

Characterisation of lysogenic phages

Phage C belonged to serological group F (table II) and had a restricted host range, lysing group-III propagating strains 6,47,85,90 and 88. Phage 67R lysed group-II strain 3C and several group-III strains and belonged to serological group A. Phage 87M lysed group-I strain 52 and group-III strains 6,54,75,83A,84,85,90,88 and belonged to serological group F. Phage 47T has already been used for routine typing in the additional set of phages reported by Vickery *et al.* (1983), and is included in table II for reference.

The influence of the presence of these phages on phage-typing patterns of MRSA strains was determined by lysogenisation studies. As expected in the following results, all lysogenised strains had lost sensitivity to the lysogenising phage.

TABLE II
Characterisation of lysogenic phages from methicillin-resistant S. aureus

Phage group of strain	MRSA strains*	Titre on propagating and test strains of phage (serological group)			
		C (F)	67R (A)	87M (F)	47T (A)
I, III	1489	2.0×10^9 /ml (5†)	1.2×10^9 /ml (5)	1.4×10^9 /ml (5)	2.1×10^9 /ml (5)
I	52	0	0	1	0
II	3A	0	0	0	3
	3C	0	1	0	1
	55	0	0	0	2
	71	0	0	0	2
III	6	5	5	5	4
	42E	0	1	0	3
	47	5	1	0	5
	53	0	5	0	4
	54	0	5	5	5
	75	0	5	5	5
	77	0	1	1	4
	83A	0	1	5	5
	84	0	4	5	5
	85	5	5	3	0
Miscellaneous	95	0	1	0	3
Experimental	90	5	1	3	1
	88	5	5	5	4
Test	2009	0	0	0	1
	8719	0	0	0	2
	42C	0	5	0	4

* Strains 29, 52A/79, 80, 81, 94, 96, 187, 3B were not lysed by these phages.

† 5 = Titre on propagating strain 1489; 4 = 10^{-1} – 10^{-2} , 3 = 10^{-3} – 10^{-4} , 2 = 10^{-5} – 10^{-6} of titre on 1489; 1 = very weak lysis; 0 = no lysis; I = inhibition reaction.

Lysogenisation of strain 1489

Strain 1489 is a MRSA strain isolated at RPAH in 1969. It is apparently non-lysogenic, because all attempts to isolate lysogenic phages from it have been unsuccessful and it was sensitive to a wide range of phages. The absence of prophage in this strain indicated that there should be no interaction between lysogenic phages and any phage isolate propagated on it. Because of the wide sensitivity of this strain to group-I and group-III phages of the Basic International Set of phages, it was used as a test strain to observe the effect of lysogenisation, with the new phage isolates, on its phage-typing pattern.

Table III shows that lysogenisation with phage C had little effect on the phage-typing pattern of strain 1489 and resulted only in reduced sensitivity to phage 85. When phage 47T was introduced, a marked loss of sensitivity to group-I and group-III phages occurred. When the double lysogen 1489(C) (47T) was tested, an additive loss of phage sensitivity resulted. Strains 1489(67R) and 1489(87M) showed loss of sensitivity to different group-III phages. The double lysogens 1489(C) (67R) and 1489(C) (87M) also showed an additive loss of phage sensitivity.

None of the lysogenised strains 1489(C) (47T), 1489(C) (67R) and 1489(C) (87M)

TABLE III
Effect of lysogenisation on the phage-typing pattern of strain 1489

Strain no. (lysogenising phage)	Effect of phages (at 100RTD) on the given strains																		
	Phage Group I					Phage Group III							Miscel- laneous phages		Experi- mental phages				
	29	52	52A	79	80	6	42E	47	53	54	75	77	83A	84	85	81	95	90	88
1489	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1489(C)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1489(47T)	-	-	-	-	-	-	-	-	-	-	-	-	I	+	+	-	-	-	-
1489(C) (47T)	-	-	-	-	-	-	-	-	-	-	-	-	I	+	+	-	-	-	-
1489 (67R)	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+
1489(C) (67R)	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-	+	+	+
1489 (87M)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
1489(C) (87M)	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+

+ = phage sensitivity; - = reduced phage sensitivity; I = inhibition reaction.

showed phage-typing patterns identical to those of the original host strains 8947, 8967 and 1987 respectively.

There was no change in the sensitivity of strain 1489 to phages 47T, 67R or 87M after lysogenisation with phage C; nor was there any change in the sensitivity of this strain to phages C, 67R or 87M after lysogenisation with phage 47T. However, there was cross immunity between phages 67R and 87M when they were introduced into strain 1489.

Lysogenisation with phage C

Only 1.6% of 748 MRSA strains isolated recently at RPAH were sensitive to phage C. All these sensitive strains belonged to phage type 83A/85/95/90/88. Two of these strains were lysogenised with phage C and the only change in sensitivity observed was a reduction in sensitivity to phage 85. Three other MRSA strains isolated earlier and of phage type 6/7/54/84/85/88 at RTD, were also lysogenised with phage C and all showed similar reduced sensitivity only to phage 85. It appears, therefore, that phage C has little effect on phage-typing patterns.

Lysogenisation with phage 47T

When strains of phage-type 83A/85/95/90/88:47T/90A were lysogenised with phage 47T, they showed loss of sensitivity to phage 88 only (table IV). However, when strains of phage type 77/83A/84/85/95/90/88:47T/56A/56C/90A were lysogenised, they showed loss of sensitivity to phage 83A, 84, 95, 90, 88 and 90A. The phage patterns of the lysogenised strains thus became 77/85:56A/56C, which was identical to that of strain 10335. Only two strains of phage-pattern 84/85/90 were sensitive to phage 47T. They were sensitive to this phage at 100RTD only. They were strain 8967 of phage-type 84/85/90:56B/56C/90A and strain 1987 of phage-type 84/85/90:90A. After lysogenisation, there was no change in their phage-sensitivity patterns.

TABLE IV

Effect of lysogenisation on phage-typing patterns of strains of methicillin-resistant S. aureus

Typing patterns with		Lysogenising phage	Changes* in phage sensitivity after lysogenisation	
Basic International Set of phages at 100 RTD	Additional phages at RTD		Gains	Losses
83A/85/95/90/88	47T/90A	47T	—	88
77/83A/84/85/95/90/88	47T/56A/56C/90A	47T	—	83A, 84, 95, 90, 88, 90A
84/85/90	56B/56C/90A	47T	—	—
84/85/90	90A	47T	—	—
84/85/90	56B/56C/90A	67R	47, 54, 75	—
83A/85/95/90/88	47T/90A	87M	—	—
84/85/90	56B/56C/90A	87M	—	—
Not Typable	Not Typable	87M	—	—

* — = No change.

Lysogenisation with phages 67R and 87M

Few strains of MRSA isolated at RPAH are sensitive to phage 67R. Strains of phage-types 83A/85/95/90/88 and 77/83A/84/85/95/90/88 and untypable strains that were studied were not sensitive, and only two strains, 8947 and 9164, of phage-type 84/85/90:56B/56C/90A were sensitive to it. These two strains were lysogenised with phage 67R and both showed slight gain in sensitivity to phages 47, 54 and 75 at 100RTD only. There was no loss in sensitivity to any of the phages tested (table IV).

All strains, including the Not Typable group, showed some degree of sensitivity to phage 87M. Strains of phage-type 77/83A/84/85/95/90/88 showed only slight sensitivity and could not be lysogenised. When strains of the other three phage types were lysogenised, there was no change in their phage-sensitivity patterns. The usefulness of this phage in strain differentiation of MRSA is being assessed.

There was no evidence, in any strain lysogenised with phages 47T, 67R or 87M, of prophage substitution of phage C.

DISCUSSION

These results indicate that the strains of methicillin-resistant *S. aureus* isolated recently at RPAH are related on the basis of their lysogenicity. Although they can be differentiated into consistent and reproducible phage-typing patterns, almost all carry the same lysogenic phage C. Strains of three phage types—83A/85/95/90/88, 77/83A/84/85/95/90/88 and untypable—carry no other detectable lysogenic phages. Schaefer *et al.* (1981) reported a similar situation in MRSA strains of phage-type 88 isolated at three hospitals in New York City. The lysogenic phage carried by their strains differs from phage C in host range, although both phages have restricted host ranges.

None of the phages isolated in this study was identical to any phage from the Basic International Set nor to any other phage previously isolated from MRSA strains (Schaefer *et al.*, 1981; Vickery *et al.*, 1983; Vickery, unpublished data). Phage C has not been detected in methicillin-sensitive strains of *S. aureus* tested in this hospital.

Lysogenisation of strain 1489 with the phages isolated from the MRSA strains isolated recently resulted in a change of phage-typing pattern, but failed to convert the pattern to that of the original host strains. This is in contrast to the situation found in the strains isolated earlier, in which the phage-typing pattern of strain 1489, after lysogenisation with the lysogenic phages from those strains, converted to that of the original host strains (Vickery, unpublished data). Moreover, lysogenisation of three early MRSA strains with phage C had little effect on their typing patterns. This result was also observed in recently isolated MRSA strains sensitive to phage C.

Strains of phage-type 84/85/90 appear to be a more heterogeneous group than the strains of other types. As well as phage C they carry other lysogenic phages. Lysogenisation with these phages had very little effect on the typing pattern of MRSA strains isolated recently except in one instance. Lysogenisation of strains of phage type 77/83A/84/85/95/90/88:47T/56A/56C/90A by phage 47T converted their phage type to 77/85:56A/56C, that of the naturally occurring MRSA strain 10335, which carried lysogenic phages identical to those of the lysogenised strains. This suggests, therefore, that the typing pattern of strain 10335 is influenced by its lysogenic phages, and that

this strain appears to have acquired phage 47T *in vivo*. Asheshov and Rippon (1959) suggested that, in type 80 strains of staphylococci, changes in phage-typing patterns may occur in nature.

The phage-typing differences between strains of patterns 83A/85/95/90/88 and 77/83A/84/85/95/90/88 cannot be ascribed to their lysogenicity, because strains of these patterns carry the identical phage C and no other prophage has been detected in them. Moreover, the untypable strains also carry this prophage that produces immunity only to phage 85.

It was noted that the untypable stains, from time to time, produced mutants which were sensitive to phages 47T and 90A. The block in phage sensitivity in the untypable strains appears, therefore to be a phenotypic character, depending on an unknown factor in the strain. It is not due to restriction enzymes because it cannot be removed by heating (Asheshov and Jevons, 1963).

A close relationship between the MRSA strains isolated recently has been demonstrated by plasmid DNA analysis. Townsend *et al.* (1983) found that recently isolated MRSA strains appeared to be different from previously reported MRSA strains. Lyons *et al.* (1984) found that although MRSA strains possessed related plasmid profiles, there was some variation in the restriction digest patterns of plasmids in strains from individual hospitals.

These studies of lysogenicity have produced no evidence that MRSA strains isolated recently are related to or may have been derived from those strains prevalent in RPAH between 1967 and 1970. Their origin is of great epidemiological interest and is being investigated further.

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