J. Hyg., Camb. (1986), **97**, 211–218
Printed in Great Britain

Plasmid profiling of epidemic staphylococci from around 1960: a comparison of epidemiological techniques

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(*Received 14 March 1986; accepted 16 April 1986*)

SUMMARY

Plasmid profiles have been established for 68 isolates of *Staphylococcus aureus* from 13 episodes of epidemic spread in hospital wards between 1958 and 1962. Despite the original lack of care in preservation of strains the profiles give, in general, the same epidemiological patterns as were established originally on the basis of phage type, antibiotic sensitivity, ward and date of isolation.

INTRODUCTION

During the late 1950s and early 1960s there was a pandemic of infection due to *Staphylococcus aureus* lysed by phages 80 and 81 and many local epidemics caused by strains lysed by other group I and by group III phages. Most strains encountered in hospitals in this era were phage typable and charts of epidemic spread were usually fairly easily constructed on the basis of phage-type and antibiotic resistance pattern.

Recently, *S. aureus* strains in hospitals are less often phage-typable and *S. epidermidis* strains present similar problems; for such strains various strategies are available including plasmid profiling. We have found this technique valuable in studies of the epidemiology of scalded skin syndrome (Dowsett *et al.* 1984), in tracing methicillin resistant *S. aureus* (Cookson *et al.* 1985) and for coagulase negative staphylococci in CAPD patients (Degener *et al.* 1986). However in the absence of other characteristics for typing strains its validity cannot be established. The availability of plasmid profiling as a tool and the existence of freeze dried staphylococci, the epidemiologic patterns of which had previously been established, prompted further study of strains originally isolated in 1958–62.

MATERIALS AND METHODS

Isolates of strains collected during long term studies of hospital infection in a London teaching hospital (hospital A) plus miscellaneous strains from a second teaching hospital (B) were available. The original epidemiology of infection and colonization by strains from hospital A was established and reported by Williams *et al.* (1962) and Noble (1962). Isolates had been subcultured on a small, but now unknown, number of occasions and had been stored on agar slopes before being freeze dried and had then been maintained in that condition. Isolates had

previously been assigned to particular outbreaks on the basis of antibiotic sensitivity, phage type and date and ward of isolation. The two collections of isolates available from hospital B lacked specific epidemiological information.

Plasmid profiles were constructed by lysing cells and separating chromosomal and plasmid DNA by gel electrophoresis (See Dowsett *et al.* 1984 for details).

Antibiotic resistance patterns were redetermined by disk test on Oxoid Mueller Hinton agar (CM 337) to penicillin (10 i.u.), tetracycline (30 μg), erythromyin (15 μg), clindamycin (2 μg), gentamicin (10 μg), neomycin (30 μg), streptomycin (10 μg), chloramphenicol (30 μg) and fusidic acid (10 μg); resistance to cadmium acetate 25 μg/ml was determined on agar plates. MIC determinations were made on blood agar base (Oxoid CM 55) incorporating concentrations of antibiotic at 5 μg/ml intervals from 1–50 μg/ml and 10 μg/ml intervals from 50–120 μg/ml. Most strains had previously been reported as resistant to penicillin, tetracycline and streptomycin but in one epidemic the pattern was resistance to penicillin, erythromycin and streptomycin and one group of isolates from hospital B were resistant to penicillin only.

Attempts were made to cure isolates of antibiotics resistance by growing them in broth containing 3 μg/ml ethidium bromide at 37 °C or at 42 °C.

RESULTS

Original epidemic details are shown in Table 1.

Plasmid profiles for isolates of each strain are shown in diagrammatic form in Figures 1 *A* & *B*. Representative gels are shown in Figures 2 and 3.

All isolates conformed to the expected pattern of resistance except where shown in Fig. 1 *A* and *B*. Strain 1 (Fig. 1 *A*) carries a large plasmid mediating penicillinase production but no other plasmids. Tetracycline and streptomycin resistance are apparently chromosomal. One isolate, 79, is tetracycline sensitive but shows resistance to erythromycin with dissociated clindamycin resistance; it also possesses a small plasmid of about the same weight as anticipated for an erythromycin plasmid but this plasmid can be cured simultaneously with the penicillinase plasmid without loss of erythromycin resistance. Since it differs chromosomally in two resistances it can be regarded as an intruder and not part of the original epidemic. Isolate 93 carries chromosomal tetracycline resistance with the same MIC as the other isolates of this strain (35 μg/ml) but has a plasmid mediating resistance to a higher level (MIC, 80 μg/ml).

Strains 2A and 2B carry a penicillinase plasmid, a tetracycline resistance plasmid and a smaller cryptic plasmid. All isolates are consistent except 64 which is now tetracycline sensitive and lacks the appropriate plasmid.

In strain 3 two isolates do not conform, 78 and 80 are sensitive to erythromycin whilst 78 is apparently chromosomally resistant to tetracycline; since both of these were isolated from the same patient they may represent intruders in the epidemic. Erythromycin resistance was not cured in isolates 77, 81 and 82.

Strains 4, 5 and 6 are consistent though small numbers of isolates are involved. Strain 6 has chromosomal tetracycline resistance at the same MIC (35 μg/ml) as strain 1 but lower than that mediated by the plasmids in other strains.

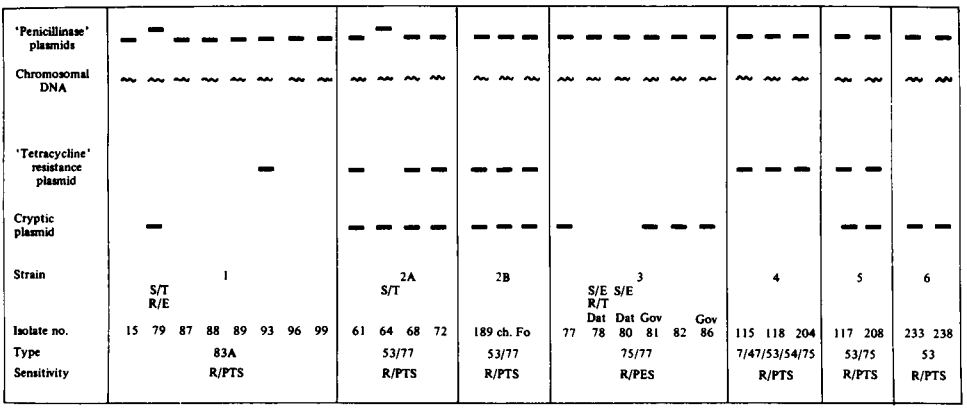
Strains 7, 9 and 10 have generally indistinguishable profiles and are consistent

Table 1. *Details of strains and of epidemic spread*

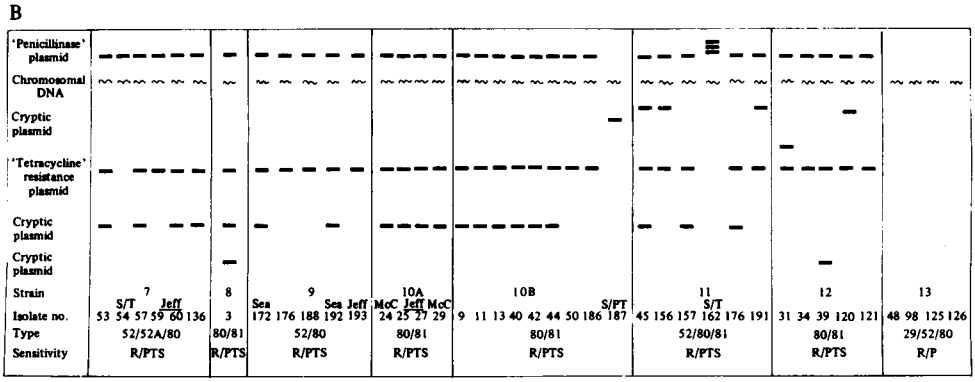
Strain	Ward in Hosp. A	Year of isolation	Total patients: Nasal colonization	Total patients: Wound 'infected'	Phage type	Resistance pattern	Comments
1	B	1959	19	12	83A	PTS	Two episodes in a single ward
2A	B	1958/9	21	6	53/77	PTS	Probably not epidemiologically
2B	P	1960	27	14	53/77	PTS	independent despite time and ward difference
3	B	1959	17	3	<u>75/77</u>	PSE	—
4	B	1960	11	0	7/47/53/54/75	PTS	—
5	B	1961	10	1	53/75	PTS	—
6	B	1959	4	3	<u>53</u>	PTS	—
7	P	1958	12	5	52/52A/80	PTS	—
8	P	1959	1	0	80	PTS	Sole isolate
9	P	1960	3	2	52/80	PTS	—
10A	P	1961	7	5	80/81	PTS	Probably a single outbreak
10B	B	1961	47	12	80/81	PTS	
11	B	1960	16	4	52/80/81	PTS	—
12	Hosp. B	1962	NK (0)	NK (6)	80/81	PTS	Miscellaneous strains present at about same time
13	Hosp. B	1962	NK (1)	NK (7)	29/52/80	P	Baby ward, outbreak of pustules

Presistance to penicillin; T, tetracycline; E, erythromycin; S, streptomycin.
NK (x), not known (number of cultures received).
Phage typing results underlined are those obtained using phage at 1000 × RTD.

A



B



Figs. 1 *A* and *B*. Diagrammatic illustration of all plasmid profiles.

except for the c 3·5 kb cryptic plasmid (isolate 54 strain 7 is sensitive to tetracycline and lacks the appropriate plasmid). The penicillinase plasmid in these strains is smaller than that of the phage group III strains (Figs. 2, 3).

Duplicate isolates from two patients SEA and MCC are consistent for all plasmids but the five isolates from patient JEF (25, 27, 59, 60, 193) are variable for the c 3·5 kb cryptic plasmid. Isolate 187 contains only a large cryptic plasmid and is presumably an intruder in the epidemic.

On the basis of plasmid profiles strain 11 would not constitute an epidemic since only isolates 157 & 176 and 156 & 191 have the same profile. Isolate 162 is most clearly an intruder but the existence of two cryptic plasmids which may or may not have been lost since isolation makes interpretation difficult.

Strain 12 may not have originally constituted an outbreak but a collection of isolates from a single hospital all of which have the same phage typing and sensitivity pattern. Strain 13 from the same hospital is entirely consistent, all isolates totally lack plasmids and the penicillin resistance is chromosomal.

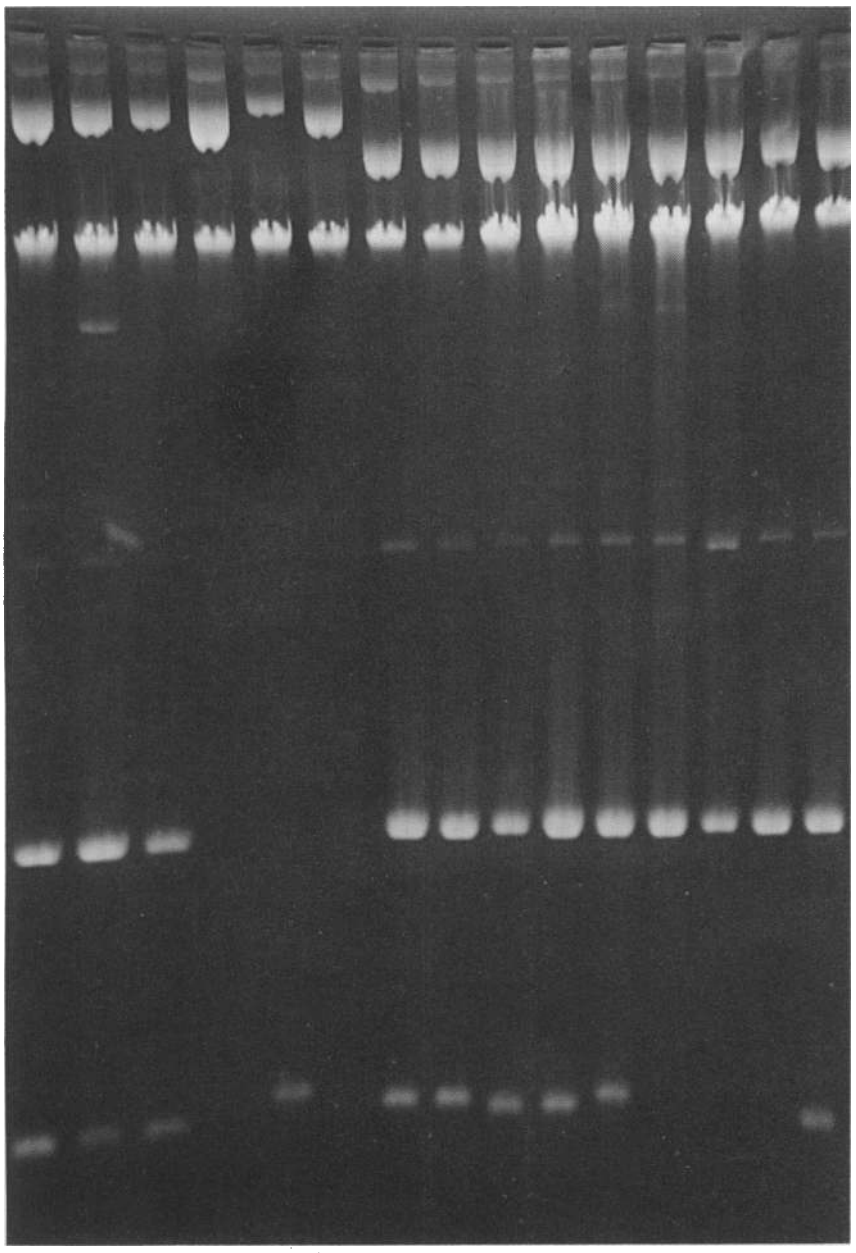


Fig. 2. Agarose gel plasmid profiles. Lanes 1–3, strain 2 isolate Ch, 68, 72; Lanes 4–6, strain 1 isolates 88, 79, 89; Lanes 7–8, strain 10A isolates 24, 25; Lanes 9–10, strain 10B isolates 9, 11; Lanes 11–13, strain 11 isolates 45, 156, 176; Lanes 14–15, strain 7 isolates 53, 57.

Plasmid identity

Plasmids migrating slower than the chromosomal band of DNA were established as governing penicillinase production and cadmium resistance by curing isolates 31, 40, 54, 79, 93, 156 and 188 with concomitant loss of these plasmids. The

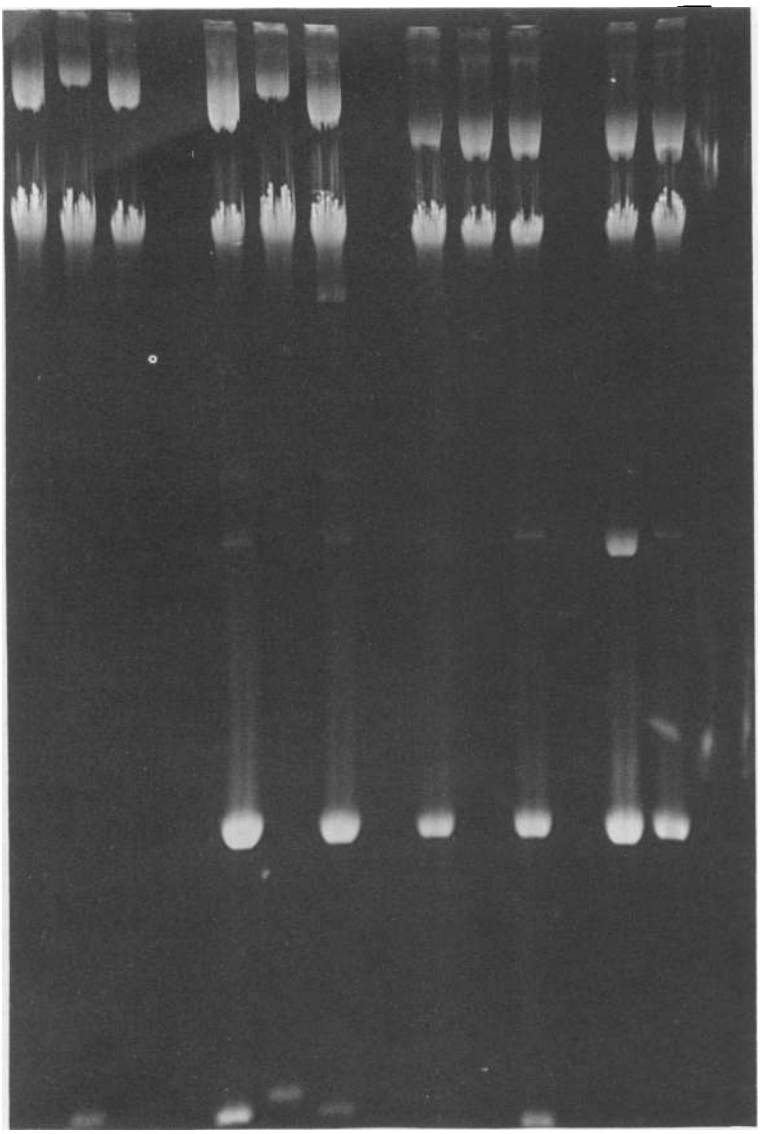


Fig. 3. Selected plasmid profiles. Lanes 1–3, strain 1 isolates 99, 79, 15, shows c 3·5 kb cryptic plasmid and larger penicillinase plasmid in isolate 79. Lanes 5–7, strain 2A isolates 61, 64, 68, shows loss of tetracycline plasmid and a larger penicillinase plasmid in isolate 64. Lanes 9–11, strain 7 isolates 53, 54, 57, shows loss of tetracycline plasmid in isolate 54 and loss of c 3·5 kb cryptic in 53 (plasmid originally present in earlier gels). Lanes 13–14, strain 12 isolate 31, 34 shows new cryptic plasmid in isolate 31 though this is at the same area as the open circular DNA from the tetracycline plasmid.

tetracycline resistance plasmid (c 4·3 kb) was indicated by its natural lack in isolates 54, 64, 162, 187, by curing in isolates 3, 40, 93, 156, 188 and Ch and by transfer of the plasmid from 93 to a known plasmid-free recipient (data not shown). However attempts to cure other tetracycline plasmids failed, even in strains known to be capable of loss. Thus in strain 2B isolate Ch was cured but

isolate Fo was not, despite examining 1267 colonies after 10 or more subcultures in broth containing ethidium bromide. Tetracycline resistance plasmids are reported to be present in high copy number which can be assumed to make curing difficult. Attempts were made to cure erythromycin resistance in isolate 77, 81 and 82 of strain 3. This was not achieved although 3779 colonies of isolates 77 were examined 844 of them after 10 successive subcultures in broth containing ethidium bromide. Comparable figures for the other two isolates were: isolate 81, 2934 (1410 after 10 subcultures) and isolate 82, 3006 (810). It was thus not possible to establish whether the c 3·5 kb plasmid in this strain mediated erythromycin resistance. The cryptic plasmid of about the same size which appears in other strains was lost during recent manipulations in isolates 53, 156 and 176, being seen in initial agarose gels but missing after exposure to ethidium bromide.

DISCUSSION

In general plasmid profiling would have given very similar epidemiological results to the original phage typing for the phage group III strains. Indeed it indicates some clear anomalies in the original designations. Given the differences in time of isolation it would also have been given the same results for the phage group I isolates if the assumption is made that the c 3·5 kb plasmid is labile and has been lost from some isolates during subculturing as has been shown to occur with isolates 53, 156 and 176. By definition a cryptic plasmid has no known markers and cannot thus be selected experimentally. The general similarity of the phage group I isolates would not originally have caused problems since the strains were isolated from different wards or in different years. Asheshov & Rippon (1959) showed that isolates typing as 80/81 could be modified to type as 52/52A/80 or as 52/52A/80/81 by lysogenization and this close relationship is presumably reflected in the plasmid profiles. One can only speculate on the role of patient Jef who, in 1958 yielded a strain of phage type 52/52A/80 Res/pen, tet, strep. from his nose (isolate 60) and gangrenous foot (59), in 1960 has a type 52/80 Res/pen, tet, strep, in an ulcer and in 1961 yields type 80/81 Res/pen, tet, strep. from his nose and an amputation stump infection.

The appearance of cryptic plamids of varying size in strains 11 and 12 has no evident explanation except the existence of plasmid transfer systems such as those recently demonstrated for the conjugative gentamicin resistance plasmid (e.g. Naidoo, 1984).

In conclusion plasmid profiling of strains of *S. aureus* approximately a quarter of a century after their isolation and storage without consideration for preservation of the plasmids yields epidemiological results generally equivalent to those arrived at previously on the basis of phage type, antibiotic sensitivity and ward and date of isolation. Our present greater knowledge of microbial genetics enables occasional clear anomalies in the original epidemiology to be demonstrated.

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