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Hospital infection caused by non-typable *Staphylococcus aureus*: application of reverse typing

By C. MARTÍN-BOURGON, S. BERRÓN and J. CASAL

Laboratorio de Referencia de Fagotipia de S. aureus, *Servicio de Bacteriología, Centro Nacional de Microbiología Virología e Inmunología Sanitarias, Majadahonda, Madrid, Spain*

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SUMMARY

Hospital infections caused by strains of *Staphylococcus aureus* non-typable (NT) by phages have occurred in three Spanish hospitals since 1981. Reverse typing allowed characterization of the strains in all three cases.

INTRODUCTION

Between 1978 and 1983 we received *Staphylococcus aureus* isolates for phage-typing from 16 episodes of hospital infection. Before 1981 a large proportion of these infections were caused by strains belonging to phage groups I–III (Martín-Bourgon, Otero & Casal, 1981); group II strains were found in four episodes, three causing neonatal skin infection and one in burns; strains belonging to the 94/96 complex have been found in three outbreaks, but in all three other strains were also involved. Groups I and 95 were uncommon.

Since 1981 three episodes of hospital infection have been due to strains of *S. aureus* non-typable (NT) by phages. This paper describes the characterization of these strains by reverse typing.

MATERIALS AND METHODS

Strains included in this study came from neonatal units of three general hospitals from different provinces in Spain (Table 1): in hospital 2 some cases were from the surgical ward. Isolates from hospitals 1 and 2 were recovered from clinical cases only, whereas in hospital 3, 11 isolates came from cases and 34 from hospital personnel. In hospital 1 a surveillance study failed to reveal clear evidence of hospital infection. Hospitals 2 and 3 sent their isolates for typing to confirm their respective outbreaks.

Isolates were confirmed as *Staphylococcus aureus* by tube coagulase and thermonuclease tests (Barry, Lachica & Atchison, 1973).

Phage typing was performed according to Blair & Williams (1961) by using the currently accepted International Set of Phages: 29, 52, 52A, 79, 80, 3A, 3C, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 81, 94, 95, 96, and two additional phages, 89 and 93, kindly provided by Dr K. Rosendal (Statens Seruminstitut, Copenhagen,

Table 1. *Phage typing of isolates from three different hospitals*

Hospital	Year	Population	No. of tested isolates	Localization of infection	Phage typing results: Number with specific pattern	Phage typing results: Typing pattern
1	1981 1982	Neonates	84	Skin*	55 29	NT Other
2	1982 1983	Neonates Surgery	59	Skin*	38 8	NT 6/42E/81 Other
3	1983	Neonates	11	Blood (4) CSF (1) Pyodermitis (4) Conjunctiva (1)	10 1	NT 95
3 (controls)	1983	Hospital staff	34	Nasal	14 20	NT Other

* Includes abscesses, wounds, infected conjunctiva and mastitis.

Denmark). These two additional phages were introduced in 1982 and were therefore not used for typing the isolates from hospital 1. Those isolates non-typable both at RTD and at 100 × RTD were further characterized by reverse typing.

Reverse typing was carried out as described by de Saxe & Notley (1979) by inducing lysates of the strains by treatment with mitomycin C and inoculating these lysates on lawns of the propagating strains and on the following test strains: 1030, W57, 42C, 2009 and 18042. Patterns were read as those strains on which the induced lysate produced any degree of lysis.

RESULTS

Phage typing revealed the presence of a large number of NT isolates in all three hospitals (Table 1), although in hospital 2 another strain was also found to be involved in the outbreak; this was typable (6/42E/81) and accounted for eight isolates. In hospital 3 all isolates from cases were NT, as were some of those from carriers.

Reverse typing of all NT isolates helped to separate them clearly into patterns. Most isolates tested – except for three of the control isolates in hospital – proved to be lysogenic and gave a positive result mainly on polyvalent strains 1030 and W57. As shown in Table 2, two different patterns were found to be prevalent in hospital 1, one accounting for 39 isolates and the other for 7. Thirty isolates from hospital 2 were found to be indistinguishable; all isolates from cases in hospital 3 were similar, whilst NT isolates from controls gave other patterns.

DISCUSSION

S. aureus isolates causing hospital infections have exhibited well-known changes in their lytic patterns and antibiotic susceptibilities in recent decades (Shanson,

Table 2. *Reverse typing of non-typable isolates from three hospitals*

Hospital	No. of NT isolates	Reverse typing: Number with specific pattern	Reverse typing: Typing pattern*
1	55	39	6/47/53/54/83A/84/1030/W57
		7	6/47/83A/1030/W57
		2	95/54/1030/W57
		2	W57/42C/2009
		5	Other
2	38	30	54/18042/1030/W57
		3	NT
		5	Other
3	10	10	95/1030
3	14	3	95/1030
(controls)		11	Other

* Pattern expressed as those strains in which the induced lysate produced any degree of lysis.

1981). In 1978, when we started typing *S. aureus* from hospitals, most strains causing hospital infection were lysed by group III phages, most frequently by 77 and 85 together with weak lysis in group I phages 29 or 79. These strains were gentamicin- and methicillin-resistant and equivalent to those described in other countries (Shanson, 1981). Since 1981 we have not seen any outbreak caused by these phage types.

In our experience, group II antibiotic-susceptible strains have produced incidental outbreaks throughout the study period and could continue to do so as a result of their ability to spread rather than as a consequence of antibiotic pressure (Parker *et al.* 1974).

The appearance of NT strains in three hospital episodes since 1981 might indicate that new strains are emerging as a substitute for the group I–III strains. Non-typable strains from hospital 1 were resistant to gentamicin and methicillin, but those from hospitals 2 and 3 were susceptible to these antibiotics. Non-typable strains causing hospital infection have recently been described in other countries (Espersen *et al.* 1982; Graham *et al.* 1980; Shanson, 1981).

The application of reverse typing is a valuable tool for the characterization of NT strains, if we consider the wide variety of strains that are included in the NT group. But looking for new phages capable of lysing these isolates is preferable, since it would avoid the use of such hazardous substances as mitomycin C and the performance of a third series of phage typing, with subsequent saving of time and material.

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