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Characterization of non-typable strains of *Staphylococcus aureus* from cases of hospital infection

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SUMMARY

A high percentage of non-typable (NT) *Staphylococcus aureus* strains was isolated in Spanish hospitals during 1984 and 1985. Several alternative methods of typing were employed to study these isolates. These were: phage-typing at 1000 × RTD, phage-typing after heat-treatment (48 °C), thermal shock (56 °C), reverse-typing and induction of additional phages. Using these methods the number of NT isolates was reduced by 60%. Best results were obtained with heat-treatment. Additional phages and reverse-typing were also useful.

A scheme for the study of outbreaks and sporadic cases caused by NT strains is proposed using the methods described.

INTRODUCTION

Non-typable strains of *Staphylococcus aureus* causing nosocomial infections in different countries have been reported by several groups (Espersen *et al.* 1982; Linneman, Mason & More, 1982; Dowsett *et al.* 1984).

In Spain, between 1978 and 1983, there has been an increase in the number of NT isolates producing hospital infection (Martín-Bourgon, Otero & Casal, 1981; Martín-Bourgon, Berrón & Casal, 1985) only group I strains were more numerous.

In this paper, we report characterization of NT strains received in our laboratory during 1984 and 1985, and analyse the feasibility of alternative phage-typing methods for typing otherwise NT strains.

MATERIALS AND METHODS

Strains

The material comprised 1473 *S. aureus* isolates from hospital laboratories sent to us during 1984 and 1985. They comprised 1175 isolates sent as part of surveillance studies in high-risk areas, and were the cause of sporadic infections; 202 had been recovered from eight hospital outbreaks and 96 constituted a group of multiple isolates from a number of patients used to evaluate the different methods of typing employed.

All strains were confirmed as *S. aureus* by coagulase and thermonuclease tests as described by Barry, Lachica & Atchison (1973).

Phage-typing

Performed according to Blair & Willians (1961) with the International Set of Phages as amended by the International Subcommittee of phage-typing of *S. aureus* in 1975.

All isolates were first studied with phages at RTD and 100 × RTD; and NT strains were further tested as follows:

Phage-typing at 1000 × RTD

Using the International Set of phages diluted at 1000 × RTD.

Phage-typing following thermal shock

Strains were grown for 4 h in Nutrient Broth at 37 °C in a shaker and then incubated at 56 °C for 2 min; they were typed with phages at 100 × RTD.

Strains 1030 and W57 which are susceptible to many phages were used to monitor phages strength without heating as reported by Lacey, Keyword & Lincoln (1984).

Phage-typing following heat-treatment

This was performed according to the method of Dowsett *et al.* (1984) by growing the strains in Nutrient Broth as previously and incubating them at 48 °C for 4 h. Typing was performed with phages at 100 × RTD. Strains 1030 and W57 were also used as a control without heat treatment.

Reverse-typing

This was carried out according to the method of de Saxe & Notley (1979) by inducing lysogenic phages with mitomycin C and testing lysates on the propagating strains of the routine phages as well as in strains 1030, W57, 2009 and 18042. Results were read using the same criteria as conventional phage-typing.

Additional phages

Sixteen phages induced from NT strains by mitomycin C treatment were used for this study. These phages had been chosen by selecting those presenting different patterns of lysis on the test strains. Four phages (50538, 50580, 50795, 50889) were finally selected because of their efficacy in lysing NT strains. These phages were purified by picking one selected plaque of lysis and propagating them by the semisolid agar method used to propagate the phages of the International Set (Blair & Williams, 1961).

RESULTS

Sporadic cases

The results obtained in the phage-typing of the *S. aureus* isolates producing sporadic infection in 1984 and 1985 show a high prevalence of NT strains (33%) in both years, group I strains (24%) were second in order of importance. The remaining phage-groups were represented by few strains, except for strains belonging to phage-group 94/96 (recently recognized as group V) that represented 16% of the total.

Table 1. *Reduction of non-typability in 345 isolates after application of several alternative phage-typing methods*

Method	1984 No. NT isolates	(%)	1985 No. NT isolates	(%)
RTD				
100 × RTD	153	(100)	192	(100)
1000 × RTD	140	(92)	—	
56 °C	121	(79)	–	
48 °C	94	(61)	127	(66)
Additional phages	69	(45)	82	(43)
Additional phages + 48 °C	59	(39)	80	(42)

Table 2. *Outbreaks of nosocomial infection produced by* S. aureus. *Characterization of NT isolates by alternative methods*

Hospital	No. isolates	Reverse-typing Pattern	No. isolates*	48 °C Pattern	No. isolates*
2	8	W57	8	—	—
3	17	95/47/54/1030/ W57/2009/18042	6	—	—
		52A/95/6/42E/47/ 1030/W57/2009/18042	5	—	—
		Other	6	—	—
5	4	95/1030/W57/2009/18042	4	95	4
6	19	47/83A/1030/W57/2009	19	—	—
7	13	53/54/1030/W57/18042	13	I–III	13
8	20	NT	20	80 (I)	20

* Number of non-typable isolates with identical pattern.

In strains isolated from highly significant clinical samples the distribution by phage-groups is very similar; NT strains represent the major group (34%) followed by group I strains (32%). These strains had been recovered from blood cultures, spinal fluid and osteomyelitis.

Outbreaks

The results of phage-typing strains isolated from patients suffering cutaneous lesions from eight nosocomial outbreaks in neonatal units in different hospitals show that in five outbreaks the causative strain was found to be non-typable. These strains were further characterized by reverse-typing and additional phages. In two other outbreaks the causative agent was typable and in the remaining outbreak there were three different strains involved, one of them being NT.

Non-typable strains

Owing to the high number of NT strains producing nosocomial infections, we explored several alternative methods in an attempt establish a scheme for their characterization.

In 1984 we studied 153 NT isolates (131 from patients, 22 from environment). Table 1 shows the progressive reduction of NT strains after the utilization

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Table 3. *Comparison of typing methods in isolates of* S. aureus *from the same patient*

				Typing-methods			
Patient	Isolates	Sources	No. same	100 × RTD	48 °C	Reverse-typing	Additional phages
1	2	Blood and catheter	2	NT	75	29/52A/80/6/47/53/54/81/1030/W57/2009/18042	NT
2	2	Blood and spinal fluid	2	NT	29	W57/1030/18042	50580/50795
3	2	Blood and wound exudate	2	NT	NT	42E/W57/1030	NT
4	3	Skin	1	NT	29	—	50580
			2	NT	NT	53/54/1030	NT
5	3	Skin	1	NT	42E/47	—	50580
			2	NT	NT	47/53/54/83A/1030/W57/2009	NT
6	3	Pusfistula	1	29/52	—	—	—
			2	NT	NT	29/52/95/42E/83A/1030/W57/2009/18042	50580/50795
7	3	Bone	1	71	—	—	—
			2	NT	NT	52A/95/42E/83A/1030/W57/2009/18042	50580/50795
8	2	Blood	2	NT	NT	1030	NT
9	6	Blood	6	NT	NT	29/95/53/54/1030/W57/2009/18042	50580
10	3	Blood	3	NT	NT	29/95/53/54/83A/1030/2009	50580 (48 °C)*
11	3	Wound exudate	3	NT	95	95/42E/1030/W57	50795 (48 °C)
12	3	Wound exudate	2	95	—	83A/1030	NT
			1	NT	95	83A/1030	NT
13	2	Exudate	2	NT	95	6/47/1030/W57/18042	50580/50795 (48 °C

14	3	Exudate	3	NT	29	6/53/54/1030/W57/2009	50580/50795
15	6	Exudate	6	NT	NT	1030/W57	NT
16	3	Wound exudate	3	NT	NT	3A/3C/71/54/1030/W57/18042	NT
17	6	Bone	6	NT	80	95/53/54/1030/W57/2009/18042	50580 (48 °C)
18	3	Wound exudate	3	NT	96	95/47/1030/W57	50795 (48 °C)
19	3	Wound exudate	3	NT	I–III	3C/71/1030/W57	50795 (48 °C)
20	3	Wound exudate	3	NT	NT	95/1030/W57/18042	NT
21	6	Exudate	3	81	—	47/53/54/1030/W57/2009/18042	NT
			3	NT	81	47/53/54/1030/W57/2009/18042	NT
22	6	Exudate	6	NT	29/75	1030/W57	50795 (48 °C)
23	3	Wound exudate	3	NT	III	3A/3C/55/71/83A/1030	50795 (48 °C)
24	3	Conjunctiva	3	NT	NT	1030/W57	50795
25	3	Conjunctiva	3	NT	NT	47/54/1030/W57/2009/	50795
26	3	Ear exudate	3	NT	NT	95/53/54/1030/W57/18042	NT
27	3	Umbilical exudate	3	NT	94	29/52/1030/W57/2009/18042	NT
28	3	Vaginal exudate	1	54/75/83A	—	47/1030/W57/18042	NT
			2	NT	54/75/83A	47/1030/W57/18042	NT
29	3	Vaginal exudate	3	NT	I–III	3A/3C/71/1030/W57	NT

* Additional phages together with heat treatment (48 °C)
NT, non-typable

of the different alternative methods. Typing at 1000 × RTD helped to characterize a small number of isolates (8·5%) but the frequent appearance of inhibition reactions made reading the results difficult. Thermal shock permitted us to type 21% of NT isolates. Heat-treatment was the most useful method, since it decreased the number of NT strains by 39%. Eight of the strains typed by this method had been previously typed by thermal shock. Finally, the application of additional phages reduced the number of NT strains to 45% of the original. This figure can be reduced to 39% when these phages are used in combination with heat treatment phage-typing. As a result of application of all the methods employed, the percentage of NT strains in 1984 decreased overall from 30 to 12%. In strains from 1985 (Table 1) there was an overall decrease of non-typability from 34 to 14%.

Table 2 shows the results of reverse-typing, heat treatment and application of additional phages in NT strains involved in six outbreaks. As can be seen, reverse-typing was useful in characterizing strains in all except outbreak 8, in which no phages could be induced, but heat treatment allowed the strains to be typed by phage 80. In outbreak 5, isolates were also characterized by heat treatment.

Evaluation of the different methods employed

During 1985 a series of strains recovered from certain patients were examined with the purpose of studying possible mixed infections. These isolates were also used for the study of efficacy and reproducibility of the several typing methods employed.

Table 3 shows the results obtained when typing these strains. Isolates have been divided into two groups: those recovered from different sites in one single patient, and isolates recovered from the same lesion of the same patient at different times. This second group comprised two types of patient: those with a mixed infection (4, 5, 6 and 7) and those with a single strain involved (8–29).

In the first group and mixed infection group, strains were characterized by heat treatment, reverse-typing and additional phages. In the remaining group there are three instances (12, 21, 28) in which heat treatment did not characterize some of the strains; these would have been considered mixed infections if no alternative methods were employed.

Additional phages

Eighty NT strains were used for the induction of phages; 16 were chosen because of their lytic potency on test strains, the lytic reaction of these phages is shown in Table 4.

All these phages were tested on 100 NT isolates. Three did not react on any strain, and seven lysed less than 10% of strains. Four were finally selected from the remainder since phage 52569 and 50538 reacted on the same strains, the latter being more effective.

A propagating strain was chosen for every phage and lytic spectra were studied. These four phages were used to type NT strains. Phages 50538 and 50889 each lysed 5 of 153 strains from 1984 and were not used in 1985. Phage 50580 lysed 38 (26%) of the 153 strains from 1984 and 71 of 192 (37%) from 1985, phage 50795 lysed 48 (32%) and 22 (11%) respectively. These phages have been included for

Table 4. *Lytic reaction of 16 phages induced by means of reverse-typing and their typing capacity in 100 non-typable isolates*

Phages	Lytic spectrum	Typing (%)
50538	95/6/54/W57/2009/18042	34
50560	95/83A/1030/W57/2009/18042	5
50558	80/6/47/83A/81/1030/W57/2009/18042	—
50580	95/47/1030/W57/18042	20
50783	95/1030/W57/2009	8
50785	71/W57/18042	—
50786	95/47/75/96/1030/W57/18042	6
50795	95/6/83A/96/1030/W57	14
50839	95/75/W57/2009	8
50889	95/75/1030/W57/18042	12
50928	71/53/1030/W57	3
50941	95/47/1030/W57	3
51186	47/53/54/1030/W57/18042	—
51832	29/52A/3A/95/6/53/1030/W57/18042	10
52339	29/3A/95/1030/W57/18042	9
52569	95/54/1030/W57/2009	14

Table 5. *Association of additional phages 50580 and 50795 with other phage-group*

	Isolates lysed by additional phages together with group						
Method	I	III	94/96	95	I–III	NT	Total
RTD	70	—	3	2	2	111	188
100 × RTD	27	6	—	—	1	77	111
48 °C	19	8	1	1	—	48	77
Total all three methods (%)	116 (62)	14 (7)	4 (2)	3 (1·5)	3 (1·5)	48 (26)	

routine purposes in our laboratory and were useful in the characterization of two of the five outbreaks caused by a NT strains, giving results compatible with those found by reverse-typing. However, they failed to lyse strains not typed by reverse-typing.

Association of additional phages with other lytic groups

In order to see if these phages could be allocated to any of the known lytic groups, they were applied together with the phages of the International Set by all the methods described above to all strains studied during 1985.

In total 188 (14%) of all strains were lysed by these experimental phages. There was a frequent association with group I phages (Table 5); 70 strains lysed by these phages at RTD were also lysed by phages belonging to group I, 27 of 111 strains not typed at RTD were typed at 100 × RTD by both additional and group I phages, and 19 of the remaining 77 NT strains were lysed by group I phages after heat treatment.

Overall 116/188 (62%) of strains lysed by additional phages were also lysed by group I phages, 7% were lysed by group III phages and 26% of the strains were typed only by the additional phages.

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DISCUSSION

Non-typable *S. aureus* producing nosocomical infections are frequent in Spain, producing sporadic infections (Martín-Bourgon, 1985*a*) and outbreaks in hospitals (Martín-Bourgon, 1985*b*; Martín-Bourgon, Berrón & Casal, 1985). Hospital outbreaks caused by NT strains have been described in other countries (Dowsett *et al.* 1984; Espersen *et al.* 1982).

In this study, we found that NT strains were involved in 32% of sporadic infections, 34% were judged as of clinical importance (bacteremia, osteomyelitis and other serious infections), as well as in five of eight nosocomial outbreaks studied.

We therefore decided to investigate other methods for typing in order to reduce the number of NT strains and to be able to characterize strains from outbreaks. Strains were studied separately in two groups: those involved in sporadic cases and those from outbreaks with the purpose of evaluating the efficacy of the methods employed.

Phage-typing at 1000 × RTD

A lot of inhibition reactions were found with this method, making reading difficult. Moreover, only 8·5% of NT strains were characterized, making the technique unsuitable for routine purposes.

Thermal shock (56 °C)

This method was described as effective in the characterization of strains by Lacey, Keyword & Lincoln (1984) but was not useful for typing strains causing outbreaks (Martín-Bourgon, 1985*a*). In our hands it permitted us to type 10% of the strains, but most of them were typed by heat treatment as well.

Heat treatment (48 °C)

This method has been described as a means of destroying restriction endonucleases and facilitating the adsorption of phages, through others state that growth of strains at high temperatures acts on capsule formation, thus affecting the susceptibility to phages (Lorian *et al.* 1985). However, Sompolinsky *et al.* (1985) reported the existence of both typable and non-typable capsulated strains which is contrary to Lorian's thesis.

In our study this method was very useful to characterize NT strains both from sporadic cases (20% reduction in 1984; 33% in 1985) and from outbreaks.

Reverse-typing

Phages induced from *S. aureus* isolates tend to act on strains lysed by many phages and show lytic effect only in a small number of the propagating strains of the International Set. For this reason it is very difficult to assess the epidemiological relatedness between strains on the basis of minor lytic reactions. On the contrary, the patterns obtained when testing isolates from outbreaks appear almost identical, making this method very useful for the characterization of strains involved in outbreaks.

In our work, reverse-typing was a very effective method, but failed in one of the

outbreaks in which no phages could be induced. Heat treatment was used with success in this case and these strains were typed by phage 80. Moreover, in two other outbreaks in which strains were characterized by reverse typing, heat treatment confirmed the relatedness of the strains.

Investigation on additional phages

The utilization of additional phages has been very important in the reduction of NT strains. In the history of the International Set new phages have been added when they were shown to type new emerging pathogens; for example the inclusion of phage 83A in order to type strains causing hospital infections in several countries (Subcommittee on Phage-Typing of Staphylococci, 1963). As *S. aureus* strains are frequent carriers of lysogenic phages that can be released by mitomycin C treatment (de Saxe & Notley, 1978), it is easy to produce additional phages.

As can be seen in our results, after induction of 100 NT strains, we found two phages that lysed 22% of strains from 1984 and 40% from 1985. These phages, when included in routine phage-typing together with the 23 phages of the International Set, showed a frequent association with group I phages. This would explain the high number of strains typed, since group I strains are very frequent in Spanish hospitals.

Evaluation of the methods employed. Proposal of a scheme for the study of sporadic cases and outbreaks

The analysis of results obtained when typing several isolates from the same patient, permitted us to compare the different methods and to look for the possibility of mixed infections.

As shown in Table 3, 4 of the 29 cases studied (4, 5, 6 and 7) presented with mixed infection.

On the other hand, the application of several different methods was useful in the identification of isolates from three cases (12, 21 and 28) which appeared as mixed infections if typed only by phage-typing at 100 × RTD; and were identical by reverse typing and heat treatment.

Reverse typing and heat treatment proved useful for the study of outbreaks, the latter being also useful for sporadic cases. Reverse-typing can also be applied as a means of obtaining experimental phages and two such phages were useful for us.

Taking into account all the above considerations we propose that when NT strains are found in sporadic hospital infections, heat treatment is the method of choice for the characterization of these strains. Additional phages would also help to reduce non-typability, but their production and selection is too tedious to be used for routine purposes.

In isolates recovered from a hospital outbreak, both reverse-typing and heat treatment give good results.

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