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Hospital infections in Spain. I. *Staphylococcus aureus* (1978–91)

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SUMMARY

This study was undertaken to determine the distribution of phage types of *Staphylococcus aureus* isolates from hospital outbreaks or sporadic cases received in our laboratory during the past 14 years. The records for 15803 isolates from 55 Spanish hospitals have been analysed.

In relation to sporadic isolates we have been able to detect the predominance of phage group I and non-typable staphylococcal strains. Since 1989, we have observed a considerable increase in hospital infection caused by methicillin-resistant *S. aureus* strains which we could differentiate in to two groups; one belonging to phage group III (6/47/54/75/77/84/85) and other groups of non-typable strains which could be classified as phage group I–III after heat treatment (29/77/84) and with similar patterns by reverse typing (6/47/53/54/75/83A/84/85/W57/1030/18042).

During 1990 and 1991, these strains have extended widely to at least six different autonomous regions creating an epidemic situation in Spain.

INTRODUCTION

Staphylococcus aureus has persisted as an important hospital and community human pathogen. According to recent data it is the fourth most frequent organism causing nosocomial infection [1]. *Staphylococcus aureus* is the most frequently reported pathogen associated with nosocomial surgical wound infection and the second most common pathogen associated with nosocomial bloodstream infections after the coagulase-negative staphylococci; all the *Staphylococcal* species together cause between 30 and 40% of such infections [2]. In relation to the medical services, *S. aureus* is the predominant pathogen in the pediatric and neonatal units [1].

Bacteriophage typing of *S. aureus* isolates is a valuable tool in the study of staphylococcal disease in man [3]. Phage typing has been successfully performed using an international set of phages for over 40 years [4], although the appearance of non-typable strains is still a problem in an epidemiological situation. Heat treatment appears useful as a method for increasing phage susceptibility and reducing the number of non-typable strains [5]. Reverse typing has also been applied to further characterize non-typable isolates [6].

We have studied 15803 isolates received during the past 14 years in the Reference Staphylococcal Laboratory (Bacteriology Service, CNMVIS, Majadahonda) in order to determine the distribution of phage types of *S. aureus* associated with hospital outbreaks and sporadic cases of infection.

MATERIALS AND METHODS

Isolates

The material comprised 15803 *S. aureus* isolates, part of a surveillance study from 55 hospital laboratories located in all Spanish autonomous regions sent to us during 1978–91. Of this figure 12535 isolates were considered to be the cause of sporadic infection, and 3268 strains from 52 outbreaks occurred in 31 hospitals.

All isolates were confirmed as *S. aureus* by coagulase and thermonuclease tests as described by Barry [7].

Phage-typing

The method of Blair and Williams [8] was used. Isolates were tested at RTD and 100 × RTD against phages of the current international set [9]. Non-typable strains were tested since 1982 by reverse-typing according to the methods of Martín Bourgon and De Saxe [6, 10] and since 1985 by phage typing after heat treatment following the methodology of Vindel and colleagues.[5].

RESULTS

Sporadic cases

The source of isolates is shown in Fig. 1. Skin isolates were the most numerous group (21%) followed by blood isolates (14·7%). Unfortunately there is a great proportion of isolates in which the origin is unknown (28·8%) because it was not given by the hospitals.

Fig. 2 shows the distribution of phage patterns of isolates from sporadic cases. The results obtained by phage-typing indicated that phage group I was the first group in importance, followed by non-typable strains. Phage groups III and V represented 14·2 and 11% respectively. Less than 10% of the strains were susceptible to group II (9·8%), mixed group (8·7%) phages or to phage 95 (6·2%).

The evolution of different phage groups involved in nosocomial infections in our country can be observed in Fig. 3. Isolates from phage group I were responsible for the majority of nosocomial infections until 1984 when the non-typable strains appeared as the more important group. For this reason, since 1985 we have employed phage typing after heat treatment. Many strains non-typable by conventional phage typing were related to phage group I after heat treatment.

Strains belonging to the mixed phage group represented an important group during 1979–80 (in this period some outbreaks caused by MRSA took place). After that, they decreased in prevalence to reach 6·7% in 1991.

Phage group III has increased its percentage during the past four years and forms a numerically important group of isolates from cases involved in nosocomial infections at present, due to the increase in MRSA strains in our country.

The rest of the phage groups showed little fluctuation in this period.

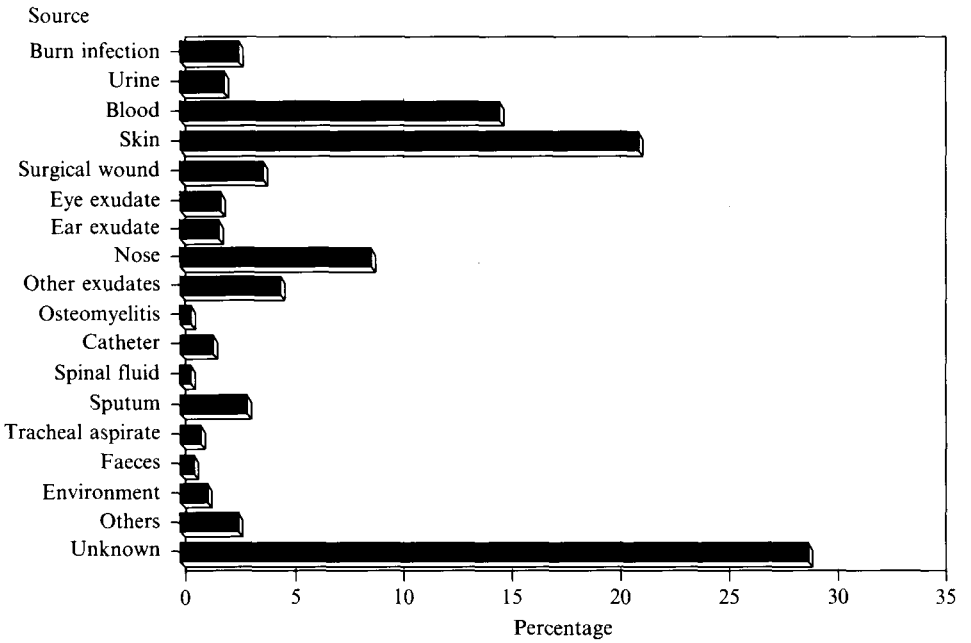

Fig. 1. Sources of *S. aureus* infections.

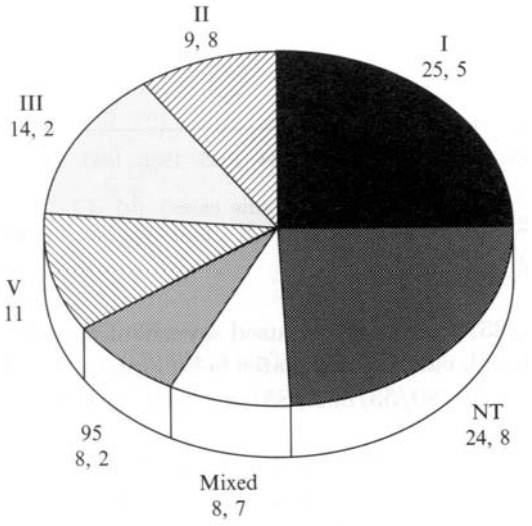

Fig. 2. Percentage of phage patterns (sporadic cases).

Outbreaks

Table 1 shows the results of 52 nosocomial outbreaks from several Spanish hospitals studied between 1978–91.

During the first period (1978–9) a high proportion of methicillin resistant (MRSA) isolates belonging to the mixed phage group I–III (29/77/85) were found to cause outbreaks. In the following years there were no outbreaks caused by MRSA strains until 1986. As seen in Fig. 4 in 1986 MRSA belonging to phage

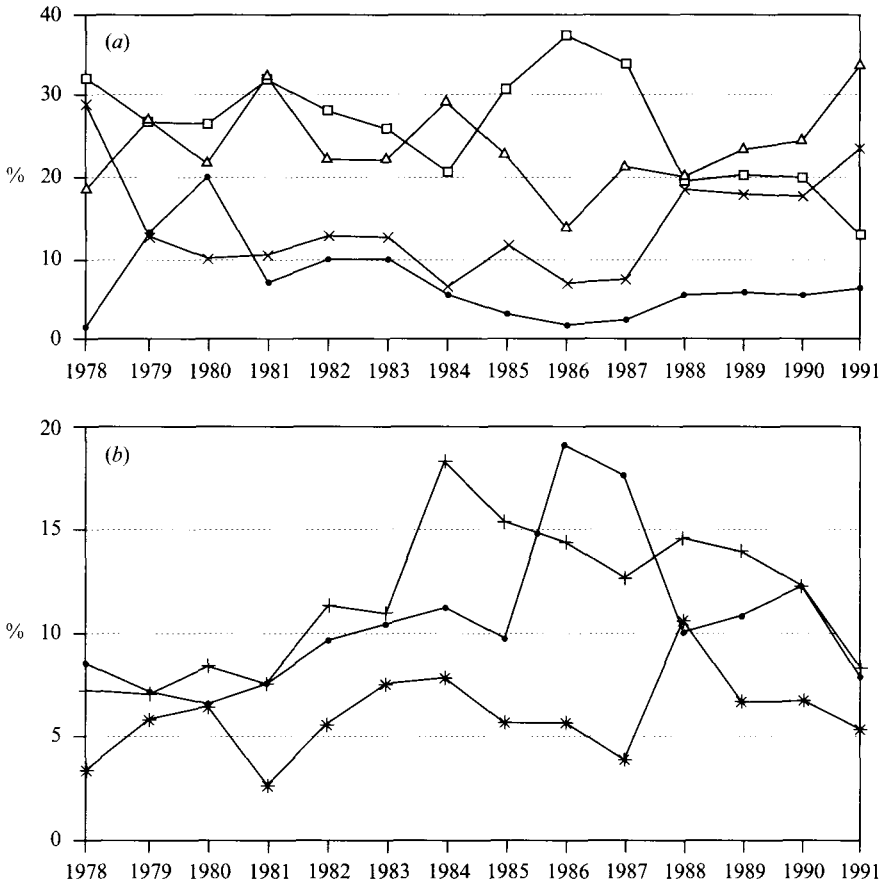

Fig. 3. Evolution of phage groups. (sporadic cases). (*a*) –□–, Phage group I; –●–, phage group I-III; –×–, phage group III; –△–, non-typable. (*b*) –■–, Phage group II; –+–, phage group V; –*–, phage 95.

groups I–III (29/54/85) and III (54) caused several outbreaks. In 1987 we studied another MRSA outbreak caused by isolates belonging to the phage group I–III by heat treatment (52A/79/80/53/83A/85) without any epidemiological relation with former strains.

During the last month of 1989 three outbreaks occurred in three hospitals. Amongst these isolates we could distinguish one group typed by group III phages (6/47/54/75/77/84/85) and another of non-typable isolates by direct phage typing but typed by group I–III phages (29/77/84) by heat treatment and with similar patterns by reverse typing (6/47/53/54/75/83A/84/85/W57/1030/18042). It was the beginning of an endemic period in Spain; during 1990–1 we analysed 22 MRSA outbreaks caused by the above mentioned strains. The source of MRSA isolates were surgical wounds and skin, both in group III (20·1%) and non-typable strains (28·5%).

In the same period of this study we also received isolates corresponding to 22 outbreaks caused by methicillin sensitive strains (MSSA) and affecting mostly neonatal units. These isolates mainly belonged to phage group II and produced epidermal lesions in babies. Again this confirms the common association made

Table 1. *Nosocomial outbreaks caused by* S. aureus

Year	Region	Unit	Phage group (phage-type)	Reverse-type	No. cases /total
1978	Pais Vasco	Neonatal	I–III* (29/77/85)	—	125/152
1979	Madrid	Burn	V (94/96)	—	6/16
		Traumatology	NT	?	5/16
			II (3A)	—	4/16
1979	Pais Vasco	Neonatal	I–III* (29/77/85)	—	69/150
			(29/47/53/54/77/83A/84/85)	—	59/150
			(29/6/42E/47/53/54/77/83A/84/85/81)	—	12/150
1980	Pais Vasco	Neonatal	II (3A/3C/55)	—	12/27
			V (94/96)	—	4/27
			I (52/52A/80)	—	4/27
1982	Madrid	Neonatal	II (3A/3C/55/71)	—	8/8
1982	Madrid	Dialysis	NT	?	4/4
1982	Pais Vasco	Neonatal	NT	54/1030/W57/18042	38/59
		Surgery	III	—	8/59
1983	Madrid	Neonatal	II (71)	—	6/19
1983	Andalucia	Surgery	V (94/96)	—	7/20
		Traumatology	95	—	5/20
1983	Rioja	Neonatal	NT	95/1030	10/11
1983	Pais Vasco	Neonatal	II (71)	—	5/5
1984	Navarra	Neonatal	I (52/80)	—	8/12
1984	Andalucia	Neonatal	NT	W57	8/20
1984	Navarra	Neonatal	NT	95/47/54/1030/W57/18042/2009	21/43
			I (80)	—	12/43
1984	Pais Vasco	Neonatal	95	—	20/79
			I (29)	—	18/79
			NT	95/6/42E/47/1030/W57/18042/2009	19/79
1985	Madrid	Neonatal	95	—	4/5
1985	Andalucia	Neonatal	NT	47/83A/1030/W57/18042	35/123
1985	Castilla-León	Neonatal	I–III (29/52A/53/83A/85)	—	13/20
1985	Rioja	Neonatal	I (80)	—	60/114
1986	Cantabria	Neonatal	95	—	8/15
1986	Andalucia	Neonatal	I–III* (29/54/85)	—	16/24
1986	Cataluña	Several	III* (54)	—	144/205
1987	Madrid	Unknown	III (54)	—	10/17
1987	Andalucia	Unknown	I–III* (52A/79/80/53/82A/85)	—	25/25
1988	Cataluña	Neonatal	NT	6/53/1030/W57/18042	9/9
1989	Madrid	Several	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	32/34
1989	Andalucia	Several	III* (47/54/77/84/85)	—	24/92
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	64/92
1989	Valencia	Neonatal	II (71)	—	9/30
1989	Madrid	Several	III* (6/47/54/75/77/84/85)	—	113/164
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	51/164

Year	Region	Unit	Phage group (phage-type)	Reverse-type	No. cases /total
1990	Aragón	Neonatal	III* (6/47/54/75/77/84/85)	—	7/59
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	48/59
1990	Madrid	Several	III* (6/47/54/75/77/84/85)	—	83/114
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	25/114
1990	Madrid	Several	III* (6/47/54/75/77/84/85)	—	25/49
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	18/49
1990	Madrid	Several	III* (6/47/54/75/77/84/85)	—	99/247
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	136/247
1990	Madrid	Unknown	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	5/9
1990	Madrid	Neonatal	II (3A/3C)	—	12/37
1990	Madrid	ICU	III* (6/47/54/75/77/84/85)	—	11/59
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	23/59
1990	Cataluña	Several	III* (6/47/54/75/77/84/85)	—	8/18
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	10/18
1990	Madrid	Several	III* (6/47/54/75/77/84/85)	—	11/165
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	150/165
1990	Madrid	Unknown	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	20/26
1990	Navarra	Unknown	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	3/5
1990	Madrid	Unknown	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	3/3
1990	Aragón	Unknown	III* (6/47/54/75/77/84/85)	—	5/18
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	3/18
1990	Andalucia	Several	III* (6/47/54/75/77/84/85)	—	39/117
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	49/117
1991	Cataluña	Several	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	126/161
1991	Andalucia	Several	III* (6/47/54/75/77/84/85)	—	12/35
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	18/35
1991	Madrid	Several	III* (6/47/54/75/77/84/85)	—	10/101
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	85/101

Year	Region	Unit	Phage group (phage-type)	Reverse-type	No. cases /total
1991	Madrid	Several	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	24/26
1991	Valencia	Several	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	5/27
1991	Madrid	Several	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	48/55
1991	Andalucia	Several	NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	231/308
			III* (6/47/54/75/77/84/85)	—	44/308
1991	Madrid	Several	III* (6/47/54/75/77/84/85)	—	39/117
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	71/117
1001	Cataluña	Several	III* (6/47/54/75/77/84/85)	—	4/10
			NT*	6/47/53/54/75/83A/84/85/W57/1030/18042	6/10

* Methicillin-resistant strains.

† Isolates implicated in the outbreak/total isolates received from the hospital.

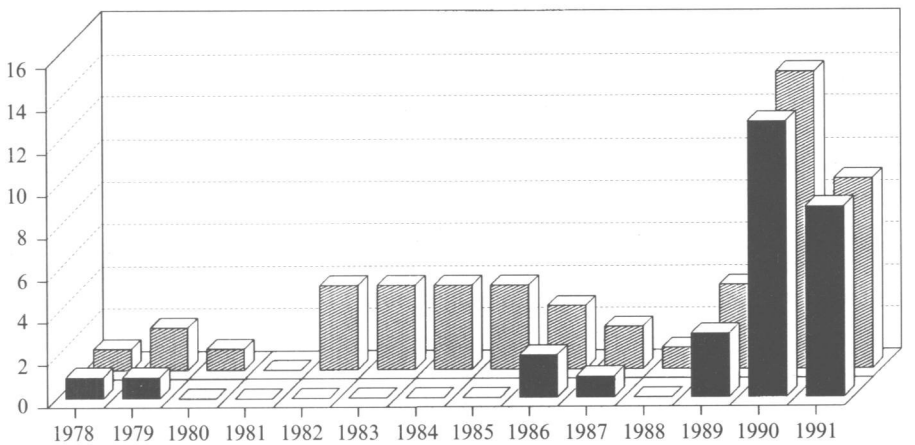

Fig. 4. Distribution of outbreaks caused by MRSA. ■, MRSA; ▨, total outbreaks.

between group II strains and skin infections [11, 12]. We also detect a group of non-typable strains which were not related to the non-typable MRSA strains since both their phage-typing patterns were different.

DISCUSSION

Phage-type characterization of nosocomial staphylococcal strains allows us to determine the *S. aureus* types causing infection as well as their evolution through the years [6]. In the present study we show the prevalence of different staphylococcal groups in Spanish hospitals.

As also happens in other countries, phage group I isolates were important in outbreaks and sporadic nosocomial infections [13].

Strains of group II appeared as epidemic pathogens as described by Parker and colleagues [14], sometimes they appeared suddenly and for short periods only [15].

Group V isolates increased their involvement in cases of sporadic infection and outbreaks, but were of little importance in outbreaks. They were associated with severe infection [16].

Isolates lysed in phage 95 were rare though they appeared in some cases causing outbreaks as described by other authors [17]. This phage group was mainly associated with severe infections.

Non-typable strains have increased in prevalence causing outbreaks as well as sporadic cases.

Strains of phage group III and mixed group I–III are frequently involved outbreaks and in endemic infections [18] associated with multiple resistance. During the last 3 years in our country, isolates of phage-group II have increased notable their involvement in hospital infections due to the spread of epidemic strains in a manner analogous to EMRSA-1 in England or the Australian MRSA [19, 20]. These strains are very difficult to eradicate from the hospital environment and cause severe infections [21–23]. These isolates were lysed by group III phages (6/47/54/75/77/84/85) and others were non-typable; this group was made typable by heat treatment giving a reaction pattern with mixed group I–III phages (29/77/84). Both types of isolate showed indistinguishable antibiotic resistance profiles and they spread in a similar manner throughout the country leading to an epidemic situation. Aparicio and colleagues [24] characterized the 29/77/84 phage-type strain and showed the existence of two variants, separable by supplementary and Fisk phage typing.

In spite of having reduced the number of non-typable isolates by application of phage typing after heat treatment, the percentage of non-typable isolates remains high. This could mean the appearance of 'unknown' new strains as an important cause of nosocomial infections and would require the search for new phages able to type these strains, or the development of new typing methods.

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