

An additional set of phages to characterize epidemic methicillin-resistant *Staphylococcus aureus* strains from Spain (1989–92)

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SUMMARY

In recent years, methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Spain have increased dramatically; in 1986 there were only 1·2% MRSA amongst all nosocomial *Staphylococcus aureus* (SA) isolates, by 1989 this percentage had risen to 44% in some hospital causing a very serious epidemic situation in the country. We have characterized these isolates by direct, reverse and Fisk phage typing and we have also looked for an additional local set of phages to help us to differentiate these strains. We have been able to differentiate an epidemic strain from other MRSA strains which cause sporadic hospital outbreaks, and we have also distinguished between some variants of the epidemic strain.

INTRODUCTION

During the past 10 years, methicillin-resistant *Staphylococcus aureus* (MRSA) strains had been causing important hospital infections around the world [1]. In Spain the percentage of MRSA isolates was formerly very low (1·2%) [2] except for some local problems [3], but late in 1989 MRSA strains caused important nosocomial outbreaks in three Spanish hospitals from two regions. During 1990 these MRSA strains spread throughout the country affecting several hospitals [4]. Since then, there has been a constant increase in the epidemic situation; and in 1991–2 MRSA strains were isolated from 31 hospitals from ten different regions.

In this paper we report the results of phage typing 3759 MRSA isolates, the selection of an additional set of phages extracted from a collection of 115 MRSA isolates from different hospitals during the 1989–91 period, and the patterns obtained after applying such phages and ten supplementary phages for MRSA [5] to a group of 1415 MRSA strains (1376 epidemic and 39 non-epidemic).

MATERIALS AND METHODS

Isolates

A total of 3759 MRSA isolates was received in our laboratory from 31 Spanish hospitals between October 1989 and December 1992.

Phage typing

Phage typing was performed by the routine method [6]. Non-typable isolates were also phage typed after heat treatment [7]. According to the results obtained with direct phage typing we selected 202 isolates to perform further studies.

Reverse typing

Prophages were induced by mitomycin C [8] from 2639 isolates non-typable by direct phage typing and from the 202 selected strains and these were matched with the propagating strains of the International set of *S. aureus* phages.

Fisk typing (Cross spotting)

Induced supernates from each of all the 202 selected strains were also spot inoculated onto lawns of all the 202 strains [9].

Antimicrobial susceptibility testing

The 202 isolates were examined using 15 antimicrobial drugs: penicillin, clarithromycin, clindamycin, rifampicin, fosfomycin, erythromycin, neomycin, fusidic acid, vancomycin, gentamicin, ciprofloxacin, cloxacillin, methicillin, bacitracin and mupirocin.

Minimal Inhibitory Concentrations (MICs) were determined by the agar dilution method [10]. The isolates were assigned to susceptibility in accordance with NCCLS criteria [11].

Selection of a local set of phages

To get an additional set of local phages, 115 isolates were selected according to their phage pattern and origin amongst the total number of MRSA isolates received in our laboratory during the period 1989–91. Prophages were induced from isolates with mitomycin C and they were spotted onto all parenteral strains. Eight phages were selected because of their high percentage of lytic capacity and discriminatory efficacy. These phages were titrated on several strains and the most sensitive was selected to propagate them. These phages were designated with numbers from 30 to 38 (except number 36). The source and origin of the lysogenic and propagating strains are shown in Table 1. They were propagated by the soft agar overlay method [12] and their lytic spectra were determined (Table 2).

Additional phages

A set of ten supplementary phages for MRSA [5] was kindly sent to our laboratory by Dr Marples (CPHL, Colindale, London). We propagated them by the soft agar overlay method [12].

A selection of 1415 MRSA isolates, including isolates from each year of the study and from all affected hospitals was phage typed with the supplementary and the local phages for MRSA strains.

RESULTS

By direct phage typing we could differentiate two groups among 3580 epidemic isolates: one group was lysed at RTD with a large number of group III phages (6/42E/47/54/75/77/84/85) and the other group was non typable but was typed after heat treatment with phages from groups I and III (29/77/84) (Table 3).

Table 1. Origin of lysogenic and propagating MRSA strains and designation of phages

Lysogenic strains			Propagating strains			Designation of phages
Number	Source	Origin	Number	Source	Origin	
8361	Blood	Madrid	8789	Sputum	Granada	30
4114	Wound	Sevilla	9302	Sputum	Barcelona	31
5715	Wound	Sevilla	6594	Blood	Madrid	32
8261	Urine	Sevilla	543	Unknown	Madrid	33
6596	Unknown	Madrid	44	Blood	Sevilla	34
8762	Skin	Granada	48	Catheter	Sevilla	35
709	Blood	Madrid	4231	Catheter	Madrid	37
2504	Blood	Madrid	1148	Exudate	Madrid	38

Table 2. Lytic spectra of local phages

Strain	Phages*							
	30	31	32	33	34	35	37	38
29	—	—	0	—	—	—	—	—
52	0	—	—	—	—	—	0	—
52A	—	—	1	1	3	1	3	1
80	1	4	1	—	2	1	0	1
2009	0	4	0	—	—	—	0	—
3A	—	—	1	1	—	1	1	1
8719	—	—	—	—	—	—	0	—
42C	0	—	0	—	—	—	0	—
42E	0	—	—	—	—	—	0	—
47	5	4	5	5	5	5	5	5
53	0	—	—	0	2	3	4	4
54	0	4	3	2	3	3	4	4
75	0	—	0	2	4	0	4	2
77	0	—	—	3	4	3	4	4

* —, indicates no reaction; 0, indicates inhibition; 1-5, indicates relative strength of reaction.

Table 3. Phage typing of MRSA isolates in Spain (1989-92)

Year	Phage-group (method)	Phage type	Number (percentage)
1989	I-III (HT)	29/77/84	147 (51.8)
	III (RTD)	6/42E/47/54/75/77/84/85	113 (48.2)
1990	I-III (HT)	29/77/84	493 (63.1)
	III (RTD)	6/42E/47/54/75/77/84/85	288 (36.9)
1991	I-III (HT)*	29/77/84	614 (79.3)
	III (RTD)	6/42E/47/54/75/77/84/85	109 (14.4)
	I-III (100 RTD)	29/42E/53/83A	51 (6.6)
1992	I-III (HT)	29/77/84	1385 (71.2)
	III (RTD)	6/42E/47/54/75/77/84/85	431 (22.2)
	I-III (100 RTD)	29/42E/53/83A	113 (5.8)
	III (RTD)	47/53/83A/84	15 (0.8)

* HT, Heat treatment.

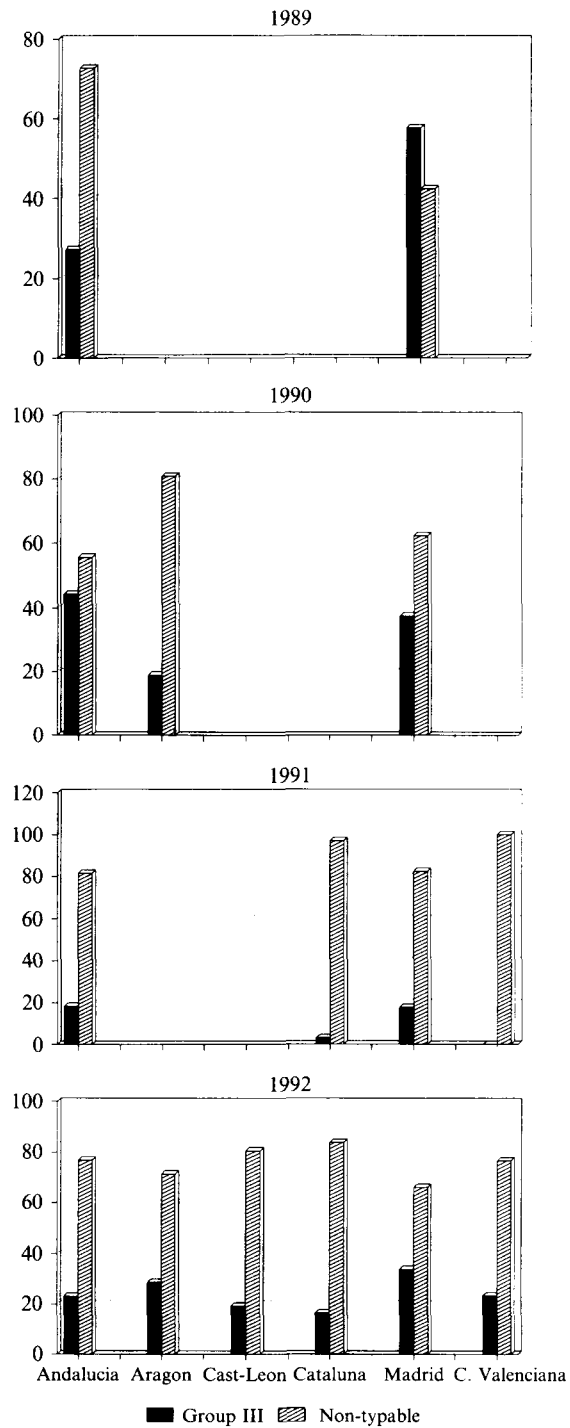


Fig. 1. Evolution of two phage-group between epidemic strains (1989–92).

Table 4. Phage typing of 1376 epidemic MRSA with additional MRSA phages

	Phage-group	
	I-III Number (percentage)	III Number (percentage)
Supplementary MRSA phages		
NT	979 (71.1)	97 (7)
618/622/626/630	110 (8)	190 (13.9)
Local MRSA phages		
NT	43 (3.1)	4 (0.3)
34	59 (4.3)	18 (1.3)
33/34/37/38	293 (21.3)	27 (2)
30/32/33/34/35/38/38	673 (48.9)	237 (17.2)
32	8 (0.6)	1 (0.1)
38	—	13 (0.9)

The distribution of these two groups of strains around the country was heterogeneous. Figure 1 shows the distribution of these two phage-groups of epidemic strains during the 1989–92 period in the most affected regions.

There were 179 isolates corresponding to two different outbreaks not related with the epidemic strains, one typed with phage group I-III (29/42E/53/83A) and the other was lysed by phages from group III (47/53/83A/84) (Table 3).

Phage typing with additional phages

A selection of 1415 MRSA isolates was studied by applying two sets of additional phages. The relationship between the patterns obtained with both sets on the epidemic strains are shown in Table 4.

After applying the MRSA supplementary set at 100 RTD we could distinguish two groups among the epidemic strains and also we differentiated a group of non-epidemic strains.

With the nine local phages obtained in our laboratory, we could differentiate clearly the non-epidemic from the epidemic strains and we also distinguished two major and two minor groups among the epidemic strains.

The evolution of the majority patterns obtained with the additional phages among the epidemic strains is shown in Figure 2.

All the epidemic strains tested, including both groups of non-typable and typable isolates showed a very similar pattern by reverse phage typing. But we could differentiate clearly the non-epidemic strains.

With the cross spotting (Fisk typing) we could differentiate at least two main groups among the epidemic strains. Again, the non-epidemic strains were clearly different.

Antibiotic susceptibility

All epidemic strains of both phage groups were resistant to penicillin (MIC > 32 mg/l), clarithromycin (MIC > 129 mg/l), clindamycin (MIC > 8 mg/l), fosfomicin (mean MIC 64 mg/l), neomycin (MIC 32 mg/l), gentamicin (MIC 64 mg/l),

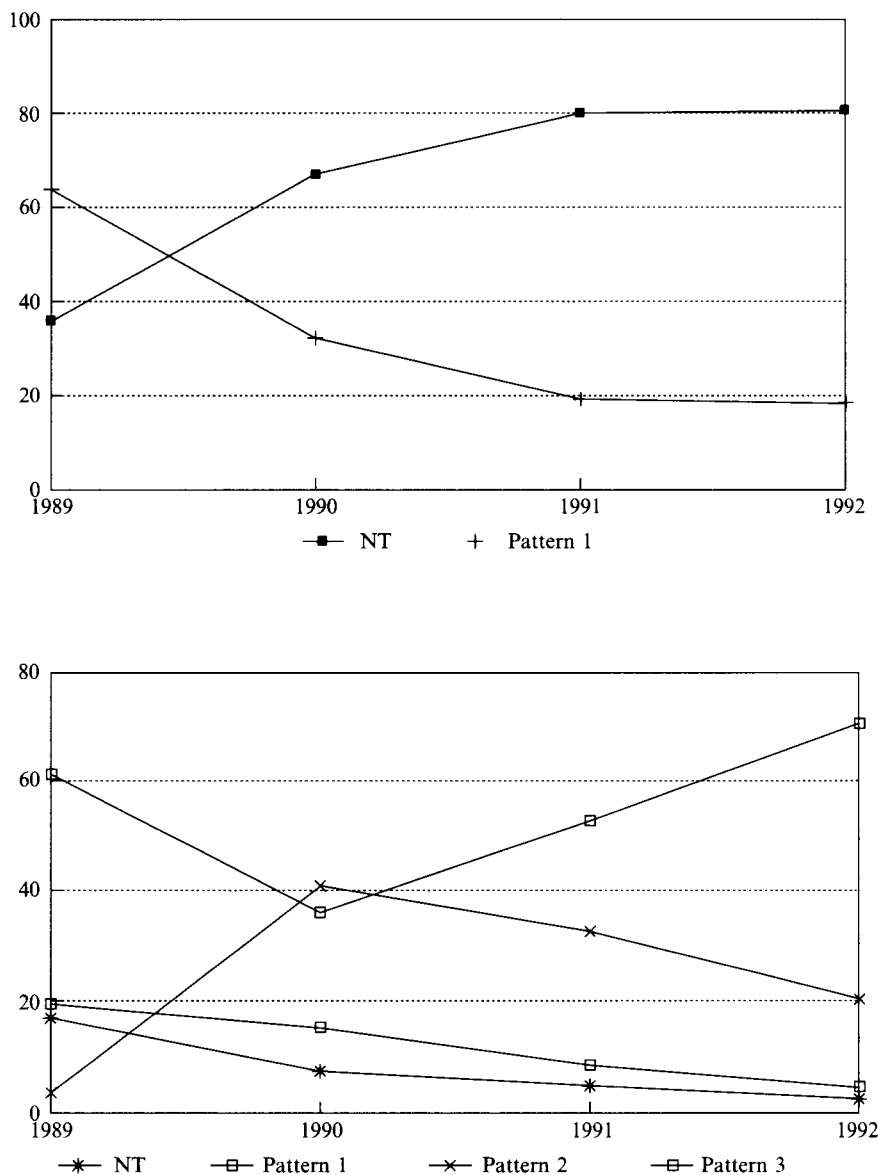


Fig. 2. Evolution of the major patterns obtained with additional MRSA phages.

bacitracin (mean MIC 25 mg/l), ciprofloxacin (MIC 16 mg/l), cloxacillin (MIC 128 mg/l) and methicillin (MIC 256 mg/l). Susceptibility to rifampicin was reduced (MIC 4 mg/l). They were uniformly susceptible to the other non-beta-lactam antibiotic tested.

The two non-epidemic strains had different antibiotic susceptibility patterns between them and with respect to the epidemic strains. One was susceptible to rifampicin (MIC 0.12 mg/l) and ciprofloxacin (MIC 0.12 mg/l) and the other was susceptible to clindamycin (mean MIC 0.06 mg/l), rifampicin (MIC 0.12 mg/l) and ciprofloxacin (MIC 0.5 mg/l).

DISCUSSION

In recent years the incidence of nosocomial infection due to MRSA has increased dramatically in many Spanish hospitals all around the country. In a survey of 74 hospitals, methicillin-resistant strains were 1.5% of the total *S. aureus* [2], in 1990 that percentage has risen to 11.0% [13]. Some hospitals have reported an incidence of 20–25% of MRSA of *S. aureus* isolates in their hospitals [14].

By phage typing we have been able to identify and differentiate between epidemic and sporadic strains involved in hospital outbreaks over the last 4 years. Also, phage typing has allowed us to analyse the evolution and dissemination of the strains that initially appeared in Madrid and Seville and further spread to other Spanish regions [15].

The fact that amongst the epidemic strains we found two different patterns with very similar epidemic behaviour and the similar patterns obtained by reverse typing lead us to think of a possible single clonal origin. To answer that question we performed a deeper study on a selection of isolates, choosing strains from all the regions and both patterns. After doing the cross-spotting phage typing we could differentiate several patterns, all of them occurring in both the major phage typing patterns, which could indicate a divergent evolution of the epidemic strains [4].

To investigate this problem further we applied a supplementary set of phages more specific for methicillin-resistant strains which were useful to identify a non-related outbreak and to differentiate two groups among the epidemic strains. Again, in both groups there were representative isolates from the two phage typing patterns. We also looked for additional local phages to increase our knowledge in relation to the evolution and differentiation of the epidemic isolates. With these local phages we found four main variants and we could discriminate the non-epidemic strains. The utility of supplementary phages to distinguish MRSA strains had been shown in different studies [16, 17] and we think that it would be useful to use an international supplementary set of phages to analyse the evolution of MRSA strains.

It further remains to apply molecular markers to complete these studies and to obtain an extensive characterization of these epidemic strains.

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