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# Development of a new set of phages as an epidemiological marker in *Staphylococcus epidermidis* causing nosocomial infections

M. M. MARTÍN-DE-NICOLÁS, A. VINDEL AND J. A. SÁEZ-NIETO*

*Laboratorio de Referencia de fagotipia de Staphylococcus aureus, Servicio de Bacteriología, Centro Nacional de Microbiología, Virología e Inmunología, Sanitarias, 28220 Majadahonda, Madrid, Spain*

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## SUMMARY

We describe the isolation of a new set of phages for typing *Staphylococcus epidermidis*. One hundred and eighty-two phages were obtained from *S. epidermidis* strains of human origin. Twelve phages were selected according to their potency and their lytic activity studied. Twenty phages of the Dean and Williams' set were also studied.

Phage-typing was undertaken at 100 × RTD, 1000 × RTD and after heat treatment at 48 °C. When the two sets of phages were compared separately similar figures were obtained. When the two typing sets were combined, the percentage of typability for the 182 bacterial strains increased to 29·1% using 1000 × RTD and to 75·3% after heat treatment.

## INTRODUCTION

Coagulase-negative staphylococci have in recent years been recognized as emerging pathogens causing nosocomial bacteraemias and other infections (1) and *Staphylococcus epidermidis* is the one most frequently isolated from such infections in intensive care units and immunocompromised patients. Treatment of these infections is often difficult because of the frequent occurrence of multiply antibiotic-resistant strains and of the problem of establishing their role in the infection. Clearly it is necessary to determine whether organisms isolated from clinical sites originate from the patients endogenous flora or from external sources. A simple and versatile typing system is needing and bacteriophage typing offers one such method for the identification of specific strains of *S. epidermidis* (2). In this paper, we describe a set of phages for *S. epidermidis* and suggest that it may find application as an epidemiological marker in nosocomial infections.

## MATERIALS AND METHODS

### *Strains*

A total of 270 strains identified in 74 hospital laboratories of Spain as coagulase-negative staphylococci were obtained. All the strains were Gram-positive cocci,

* Author for correspondence.

Table 1. *Sources of 182 isolates of* S. epidermidis *used in the phage-typing study and 88 isolates of other coagulase-negative species*

| Source | *S. epidermidis* | *S. haemolyticus* | *S. hominis* | *S. simulans* | *S. warneri* | *S. capitis* | *S. xylosus* |
|---|---|---|---|---|---|---|---|
| Blood | 51 | 5 | 8 | 4 | 3 | 1 | — |
| Catheter | 21 | 2 | 2 | 1 | — | — | — |
| Wound exudate | 34 | 12 | 5 | 5 | 2 | — | 4 |
| Eye exudate | 20 | 2 | — | — | 1 | — | — |
| Nasal exudate | 13 | 2 | — | — | — | — | — |
| Urine | 13 | 2 | 1 | 5 | 1 | — | 3 |
| Abcess | 4 | — | 1 | — | — | — | — |
| Spinal fluid | 4 | — | 1 | — | — | — | — |
| Ear exudate | 3 | — | — | 1 | — | — | — |
| Sputum | 2 | — | — | — | — | — | 2 |
| Others | 17 | 4 | 1 | 3 | 3 | 1 | — |
| Totals | 182 | 29 | 19 | 19 | 10 | 2 | 9 |
| % | 67·5 | 10·7 | 7·0 | 7·0 | 3·7 | 0·7 | 3·3 |

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Table 2. *Lysogenic bacterial strains and propagating strains of* Staphylococcus epidermidis *and phage designations*

| Lysogenic bacterial strain (source) | Propagating strain (source) | Phage designation |
|---|---|---|
| 89904 (eye exudate) | 89925 (blood) | 89904 |
| 89954 (eye exudate) | 90418 (nasal exudate) | 89954 |
| 90319 (blood) | 90343 (blood) | 90319 |
| 90338 (blood) | 89943 (eye exudate) | 90338 |
| 90340 (wound exudate) | 89939 (vagina exudate) | 90340 |
| 90341 (blood) | 90510 (wound exudate) | 90341 |
| 90352 (skin) | 89939 (vagina exudate) | 90352 |
| 90502 (blood) | 89940 (blood) | 90502 |
| 90509 (wound exudate) | 90374 (rectum) | 90509 |
| 43763 (urine) | 89939 (vagina exudate) | 43763 |
| 43764 (blood) | 89939 (vagina exudate) | 43764 |
| 43785 (blood) | 90319 (blood) | 43785 |

Table 3. *The lytic spectra of the twelve phages*

| Bacterial strains* | Phages† | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 15 | 0 | 5 | 4 | 5 | 3 | 3 | 0 | 5 | 0 | 4 | 3 | 3 |
| 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 71 | 3 | 3 | 5 | 5 | 5 | 5 | 3 | 5 | 5 | 5 | 5 | 5 |
| 82 | 0 | 0 | 1 | 3 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 |
| 155 | 5 | 5 | 4 | 5 | 5 | 4 | 3 | 5 | 5 | 5 | 5 | 5 |
| 157A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 165 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 275 | 3 | 3 | 3 | 4 | 3 | 4 | 3 | 5 | 5 | 5 | 4 | 3 |
| 275A | 2 | 1 | 2 | 3 | 2 | 3 | 3 | 4 | 4 | 5 | 4 | 3 |
| 456 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| 459 | 5 | 4 | 4 | 2 | 4 | 5 | 4 | 5 | 5 | 5 | 4 | 4 |
| 471A | 0 | 0 | 0 | 5 | 2 | 0 | 5 | 3 | 5 | 2 | 0 | 3 |
| A6C | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A9C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B1 | 0 | 0 | 0 | 2 | 0 | 0 | 5 | 2 | 1 | 0 | 0 | 0 |
| RG | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

† A, 89904; B, 89954; C, 90319; D, 90338; E, 90340; F, 90341; G, 90352; H, 90502; I, 90509; J, 43763; K, 43764; L, 43785.

* Dean–Williams propagating strains.

Numbers, grades of lysis. None = 0 to confluent = 5.

produced catalase and did not have coagulase activity by the tube test using rabbit plasma. Coagulase-negative strains which produced yellow or rubbery white colonies were tested for acid production from glucose anaerobically and for resistance to lysostaphin (3). The identifications of most of the coagulase-negative clinical isolates were confirmed by the method of Kloos and Schleifer (4, 5). A total

Table 4. *Frequencies of phage patterns among 182 strains of* Staphylococcus epidermidis

| Phage pattern | No. of strains |
|---|---|
| (*a*) With the 'Dean and Williams' set | |
| 37 | 27 |
| 37, 48 | 26 |
| 456 | 11 |
| 27, 456, 459, 471A, A6C, B1 | 10 |
| 456, 459 | 6 |
| 27, 28, 28A, 82, 155, 157A, 257A, 456, 459, 471A, A6C, B1 | 3 |
| 48, 456 | 2 |
| 28A | 2 |
| Other combinations | 31 |
| Non-typable strains | 64 |
| (*b*) With the new set | |
| All phages | 13 |
| 43763, 43785 | 6 |
| 43785 | 4 |
| 90509, 43763, 43785 | 4 |
| 89954 | 3 |
| 89904, 90319, 90340, 90341, 90352, 90509, 43785 | 3 |
| 89904 | 3 |
| 89904, 90352, 43785 | 2 |
| 43763, 43754, 43785 | 2 |
| 90352 | 2 |
| Other combinations | 40 |
| Non-typable strains | 100 |
| (*c*) With the combined set | |
| 37 | 19 |
| 37, 48 | 11 |
| 15, 37, 48 | 9 |
| 43785 | 6 |
| 456 | 6 |
| All except 15, 28A, 165 | 5 |
| 43763 | 2 |
| 90341, 90352, 43763, 43764, 43785, 157A | 2 |
| 89904, 27, 456 | 2 |
| All except 28A, 165 | 2 |
| 15, 37 | 2 |
| 90319, 90338, 90340, 90341, 90352, 43764, 43785 | 2 |
| 27, 275, 456, 471 | 2 |
| Other combinations | 67 |
| Non-typable strains | 45 |

of 182 isolates were confirmed as being *S. epidermidis*. The origins and species of the isolates are shown in Table 1.

*Reverse-typing*

This was carried out according to the method of De Saxe and Notley (6) by inducing lysogenic phages with mitomycin C and testing lysates on the propagating strains of *S. epidermidis*.

Table 5. *Frequencies of reactions of the different test phages (mixed set)*

| Phage | No. of strains (%) | Phage | No. of strains (%) |
|---|---|---|---|
| 37 | 65 (35·7) | 90509 | 36 (19·7) |
| 456 | 58 (31·8) | 90502 | 30 (16·4) |
| 43785 | 55 (30·2) | 155 | 28 (15·3) |
| 27 | 48 (26·3) | 89904 | 26 (14·2) |
| 90352 | 45 (24·7) | 28 | 25 (13·7) |
| 48 | 45 (24·7) | 90341 | 24 (13·1) |
| 471A | 42 (23·0) | 157A | 24 (13·1) |
| 43763 | 40 (21·9) | 89954 | 23 (12·6) |
| B1 | 39 (21·4) | 15 | 22 (12·0) |
| 43764 | 38 (20·8) | 275 | 20 (10·9) |
| 90319 | 37 (20·3) | 28A | 10 (5·4) |
| 90338 | 36 (19·7) | 165 | 3 (1·6) |
| 90340 | 36 (19·7) | | |

*Selected new phage set and the set of 'Dean and Williams'*

The phages induced from 182 strains by mitomycin C treatment were screened for this study. Of these, 12 phages were finally selected because of their lytic efficacy. Sources, propogating strains and designations are given in Table 2. The phages were propogated by the semisolid agar method (7). The lytic spectra were studied on the propagating strains of the 'Dean and Williams' set (Table 3). The 12 phages are referred to as the 'new' set.

We also used the set of phages known as the Dean and Williams (DW) set. This consists of 10 phages originally isolated by Dean and colleagues (8) for the typing of coagulase-negative staphylococci, 9 phages characterised by Verhoef and co-workers (9), and one (phage B1) isolated by De Saxe and Notley (6).

They were sent to us by Dr Rosdahl (Statens Serum Institute (Copenhagen) and were propogated and their lytic spectra studied.

*Phage-typing methods*

Phage-typing at 100 × RTD, 1000 × RTD and following heat treatment (48 °C), was performed according to methods previously described (10).

## RESULTS

The reproducibility of results with our set of 12 phages and the 20 phages of the 'Dean and Williams' set was established by testing 10 randomly selected strains of *S. epidermidis* 3 times on 3 consecutive days using freshly prepared cultures on each occasion. The results showed satisfactory reproducibility. Because the activity of the phages at 100 × RTD and at 1000 × RTD was so similar, the 100 × RTD system was abandoned and only 1000 × RTD typing following heat treatment was used for subsequent tests.

*DW set*

The proportion of non-typable strains with these phages was 76·5% and 70·9% at the 100 × RTD and 1000 × RTD respectively. After the application of heat treatment (48 °C) the proportion decreased to 35·2%.

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Table 6. *Phage typability of* Staphylococcus epidermidis

| | Strains untypable | Typable strains reactions: All | 6* | 5† | 4† | 3† | 2‡ | 1‡ | Total |
|---|---|---|---|---|---|---|---|---|---|
| DW set | 64 (35·1%) | 118 | 27 (14·8%) | 2 | 5 (8·7%) | 9 | 33 (41·2%) | 42 | 182 |
| New set | 100 (54·9%) | 82 | 31 (17·0%) | 5 | 5 (14·8%) | 17 | 11 (13·1%) | 13 | 182 |
| Combined set | 45 (24·7%) | 137 | 50 (27·4%) | 6 | 10 (17·0%) | 15 | 21 (30·7%) | 35 | 182 |

* Long pattern reactions.
† Indetermined pattern reactions.
‡ Short pattern reactions.

*New set*

With the 12 phages in the new set, the proportion of non-typable strains was also reduced after heat-treatment from 75·3 and 54·9%. In general the range of activity of the phages was low but where organisms were susceptible, lysis was usually confluent.

*Combined set*

Thirteen phages were selected from the DW set. As the five phages (456, 459, 471A, A6C and B1) almost always produced the same lytic effect on the strains, only phages 456, 471A and B1 were selected. The remaining 10 were chosen because of their wider range of activity compared to the rest.

When a combined set of our 12 phages and the 13 of the DW set was applied an even greater reduction of non-typable strains from 70·9 to 24·7% after heat-treatment was obtained.

*Phage patterns*

Phage patterns with the DW and new sets, and with the combined set are presented in Tables 4 (*A*, *B* and *C*).

In Table 5 the frequencies of reactions with the different phages are presented.

In Table 6 the frequencies of phage patterns are recorded. The patterns found amongst the typable strains were divided into three sets; long, indeterminate and short patterns.

## DISCUSSION

In our study the most successful technique of characterizing strains was to perform the phage typing at 1000 × RTD following heat treatment, which is similar to our recent experience with *Staphylococcus aureus* (11). The rationale has been ascribed to the destruction of restriction endonucleases with resulting facilitation of the absorption of the phages (12). Lorian and colleagues (13), however, consider that growth at high temperature depresses capsule formation affecting susceptibility to phages. However, Sompolinsky and co-workers (14) reported the existence of both typable and non typable capsulated strains thus casting some doubt on that view.

De Saxe (6) using the DW set of phages at 100 × RTD achieved 57·6%

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typability on strains isolated in the UK compared with 23·5% which we achieved with Spanish strains using the same DW set. Clearly sets devised so far do not provide the same international coverage as does the standard set available for *S. aureus* (15). Such variations are well recognized (16) and are associated often with particular geographical locations. Further when phage patterns are divided into short, indeterminate, and long patterns according to the criteria of Richardson and Marples (17) a greater proportion of the more desirable short patterns were found with the DW set than either the new or the combined sets.

It has not been possible to attempt to relate strains with similar phage patterns functionally as so many hospitals were involved and no epidemiologically related isolates were recorded. We are, however, prepared to supply our phages and propogating strains to those who may be interested to examine their own isolates particularly where likely common sources may have been identified.

It is hoped that this characterized phage set may provide further discriminatory powers in the identification of particular strains of *Staph. epidermidis*. Clearly, however, the aim must still be to increase the proportion of typable strains and at the same time to reduce if possible the number of phages needed to provide adequate distinction between organisms.

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