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A STUDY OF HOSPITAL ACQUIRED STAPHYLOCOCCAL DISEASES BY MEANS OF PHAGE TYPING IN RIBEIRÃO PRETO, SP, BRAZIL

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SUMMARY

In order to base preventive measures against hospital acquired staphylococcal diseases on epidemiological evidences derived from microbiological investigations, a laboratory designed for phage typing of *Staphylococcus aureus* was established in Ribeirão Preto, SP, Brazil. This study pertains to incidents involving 95 consecutive cases of hospital-acquired staphylococcal diseases in a General University Hospital, 1966. Fifty-four of these strains could be submitted to phage typing with results as follows. Phage patterns of the "52, 52A, 80, 81" Complex were responsible for 24 cases (44%). Group III phage patterns, for 13 cases (24%), the majority of which with reactions only to 83A, 84, 85 phages. Overlapping patterns between the mentioned complex plus Group III phages, for 4 cases (8%). Group II phage patterns, for 6 cases (11%). The remainder 7 cases (13%) had untypable strains. Furthermore, a significant difference (at 5% level) of distribution of phage patterns was observed in two groups of the strains involved in the cases of staphylococcal diseases: 1) in-patients (54 cases, as above referred), and 2) out-patients (145 cases). A third group formed by 79 healthy carriers among professional staff (97 strains) showed an intermediate situation.

INTRODUCTION

In a previous investigation [7], strains of *Staphylococcus aureus* isolated in 1966 from inside and outside hospital acquired staphylococcal diseases in a general school-hospital, were studied with regard to: 1) susceptibility to mercury-bichloride, according to MOORE [5], and 2) sensitivities (antibiogram) to four basic antibiotics, according to BARBER & BURSTON [2].

In the present retrospective epidemiological study, the majority of the same strains were phage-typed at Ribeirão Preto, São Paulo, Brazil. In a forthcoming paper [8], we shall compare this new data on the strains with the above mentioned previous criteria adopted for their characterization.

MATERIALS AND METHODS

Strains — The 404 strains used for the previous investigation [7] were preserved by freezing at −18°C to −20°C their cultures on defibrinated sterile normal rabbit blood [6], in 1966. By 1970-1971, 296 strains, or more than 70% of the initial number survived, and these cultures were then phage-typed.

Staphylococcus aureus bacteriophages — One of us (C.S.-V.) brought the seeds of phages, propagating strains and standard test strains from the laboratory of Dr. Peter Byrd Smith (CDC, Atlanta, Ga.) to Ribeirão Preto, São Paulo, in June 1969. In the ensuing year propagations and determination of lytic spectra of the phages were done

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according to recommended methods [3]. Samples of the first batches were sent to the International Staphylococcus Reference Laboratory (Colindale, London) to be cheked by courtesy of Dr. Elizabeth H. Asheshov. The whole range of 22 phages of the International Basic Set (*i.e.*, 29, 52, 52A, 79, 80, 3A, 3C, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 42D, 81, 187) were used to classify our strains.

To minimize the possibility of contamination in the process of propagation of the phages we used Fernbach flasks instead of large size Petri dishes, collecting the growth by means of a 50 ml pipette.

The viability of the staphylococcus cultures was standardized by two consecutive subcultures in broth: one overnight culture followed by a second one from a very small inoculum from the first. That second culture was used after 4 to 6 hour incubation either for propagation or for phage-typing.

This was done according to recommended methods [3]. The RTDs keep well at least for four weeks at 4°C.

The phage-type is defined in correlation to the phages which produced a strong reaction (at least more than 50 plaques, or a two plus reaction). The strains which did not react significantly in that way to any one of the phages at RTD were submitted again to the whole range of phages (except 83A, 84, 85, according to the rules) at a concentration of 1000 × RTD.

RESULTS

The suitability of the International Basic Set of phages for typing our 1966 strains was verified: the proportion of untypable strains remained within acceptable range (20% at most); the majority of strains were typable at RTD and were not overlapping (*i.e.*, they did not react with more than one Group of phages).

The "52, 52A, 80, 81" Complex, particularly if found alone in the pattern, represented the main incriminating pattern for hospital acquired staphylococcal diseases. The Group III patterns came next, particularly when phages 83A, 84, 85 are present (Table I).

A difference (at the 5% level of significance) of phage patterns distinguished between two major groups of cultures in this study: 1) isolates from inside hospital acquired staphylococcal diseases, and 2) isolates from outside hospital acquired staphylococcal diseases; the strains from the professional staff healthy carriers made up a third intermediate group (Table I).

A chronogram (Fig. 1) presents the 1966 incidence studies in a concise form.

DISCUSSION

Typing at higher concentration of phages — Shortly after finishing the phage typing of our strains we were informed of the last recommendation (1970) of the International Subcommittee on Phage Typing of *Staphylococcus aureus* that 100 × RTD be used instead of 1000 × RTD. This, in order to avoid: 1) inhibition reactions, and 2) too complex patterns, with possibly no reduction of the proportion of phage typable strains.

Untypable Strains — A small overall proportion of untypable strains (8%) was verified. The highest proportion (14%) was observed among healthy carriers, which was nearly three times higher then that for patients (5%). The out-patients showed a particularly low proportion (2%), nearly six times lower than that for in-patients.

Phage-Type patterns — The 272 strains (92% of 296) which actually reacted with the phages in the significant two plus level were distributed among as many as 117 patterns, a degree of differentiation which is in a accord with general experience [9]. The reduction to a lesser number of approximately similar patterns is possible, however, by identification of presumably related strains which, although differing by one, two, or, in some special cases of epidemiological situation, by even three reactions, could be considered the same [3].

Uni-Group and Overlapping Patterns — From the 272 typable strains, 198 (72%) exhibited varied patterns, which involved phages belonging to only one of the Phage Groups, whereas the remainder 76 strains (28%) were characterized by patterns with overlapping Groups.

TABLE I

Prevalence of Phage Type Patterns among Inside and Outside Hospital Acquired Staphylococcal Diseases and Professional Staff Healthy Carriers (296 strains)
General Hospital, Ribeirão Preto, SP, Brazil, 1966

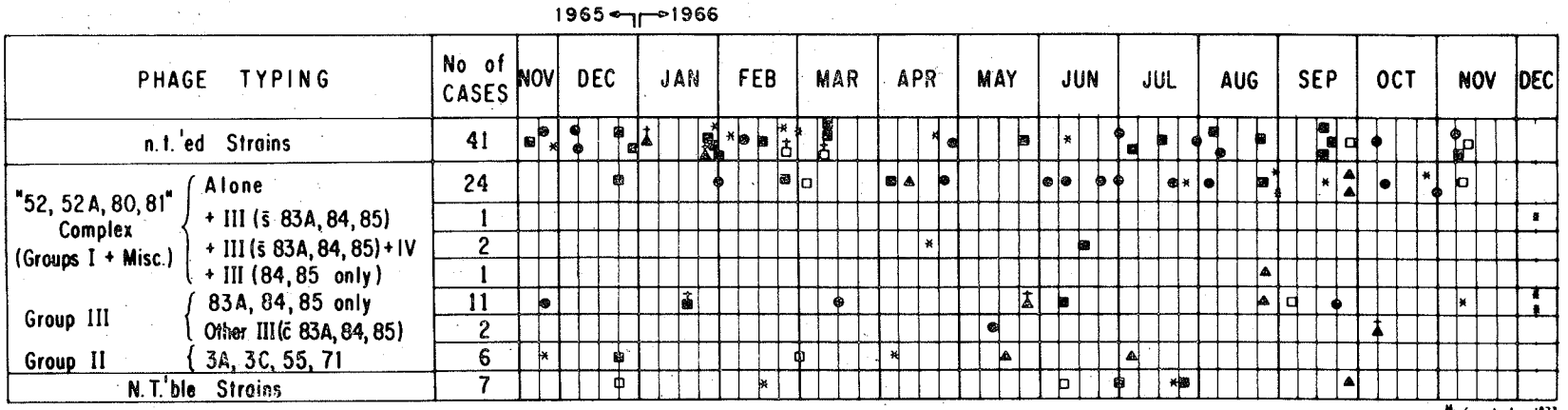
Phage Patterns		In-Patients (*)		Carriers		Out-Patients (*)	
		No. of cases	%	No. of strains (**)	%	No. of cases	%
"52, 52A, 80, 81" Complex		24	44.0	30	31.0	59	41.0
Overlapping patterns: 52, 52A, 80, 81 Complex plus III	$\overline{s}$ 83A, 84, 85	1	2.0	7	7.0	12	8.0
	$\overline{s}$ 83A, 84, 85 plus IV	2	4.0	7	7.0	5	3.0
	83A, 84, 85 only	1	2.0	1	1.0	3	3.0
III	83A, 84, 85 only	11	20.0	12	12.0	10	7.0
	other III ($\overline{c}$ 83A, 84, 85)	2	4.0	1	1.0	12	8.0
	other III ($\overline{s}$ 83A, 84, 85)	—	0.0	13	14.0	5	3.0
	other III ($\overline{s}$ 83A, 84, 85) plus II	—	0.0	2	2.0	1	1.0
II: 3A, 3C, 55, 71		6	11.0	10	10.0	26	18.0
Unt.'ble Strains		7	13.0	14	15.0	12	8.0
Total		54	100.0	97	100.0	145	100.0

(*) significant difference, 5% level
(**) corresponding to 79 carriers since 18 carriers had staphyloccci on both nose and throat

Legend: Roman Numerals: respective Phage Groups
$\overline{c}$ = with
$\overline{s}$ = Without
Unt.'ble = Untypable

Fig. 1 (see Table I)

CHRONOGRAM OF 95 CONSECUTIVE HOSPITAL ACQUIRED STAPHYLOCOCCAL DISEASES IN A GENERAL HOSPITAL(*)



(*) RIBEIRÃO PRETO MEDICAL SCHOOL HOSPITAL, UNIVERSITY OF SÃO PAULO (Ribeirão Preto, São Paulo, Brazil)
(300 BEDS; 9,250 IN-PATIENTS IN 1966)

Legend (in parenthesis: respective amount)

n.t.'ed = not typed (41) — s̄ = without — † = Death of Patient (7) — □ = Dysentery (10) — ▲ = Respiratory Infection (12)

N.T.'ble = Non-Typable (7) — c̄ = with — ■ = Diarrhœa (28) — ● = Surgical Infection (24) — ✱ = Skin Condition (21)

Out of the latter strains, the overlapping "Group I — Miscellaneous" ("52, 52A, 80, 81" Complex) is the most important in frequency and role (Table I).

Typability at RTD and 1000 × RTD — Out of the 272 typable strains, 164 (60%) were so at RTD, whereas the remainder were typed at 1000 × RTD only. The strains typed at RTD were uni-Group in the proportion of 78%, whereas the ones typed at 1000 × RTD only were uni-Group in the proportion of 64%.

Therefore, typing at 1000 × RTD gave more overlapping, consequently more complicated patterns, and the inhibition reactions weren't too rare, all as would be expected.

Professional Staff Healthy Carriers — Eighty-nine members of the professional staff were examined for carriers, 79 of them were positive (89%). From these 79 carriers, 18 (23%) carried staphylococci both in the nose and the throat. Nine of these (50%) had in the nose and throat entirely identical phage-type strains. Six carriers (33%) showed significant differences between the phage-types of their two strains. The remainder 3 carriers (17%) couldn't have all their strains phage-typed.

The 1966 Incidents — When we first started to study these incidents we thought it possible to divide the Hospital into separate "epidemiological units" [7].

We were convinced, however, that such separate units did not correspond precisely to the prevailing epidemiological conditions. It was a small hospital of 300 beds with only one surgical center, and a registration of 9,250 in-patients in 1966, with a free circulation of students, physicians, residents, nurses and auxiliary personnel as well. We shall consider, at this time, the whole hospital as only one "epidemiological unit" (Table I, In-patients; Fig. 1).

In 1966 there occurred 95 hospital-acquired staphylococcal diseases, with 7 deaths. Only 54 (57%) of the strains thus involved could be submitted to phage-typing, and 7 (13%) were untypable.

The strains from three of the seven patients (all children) who died, were phage-typed: two, type 84/85; and the third was 53/83A/84.

Many incidents, *i.e.*, cross-infections [10], may be visualized in 1966 (Fig. 1).

In Table I the phage patterns found among in-patients may be seen. According to decreasing importance in terms of frequency we had, first, the "52, 52A, 80, 81" Complex alone as the cause of 24 cases (44%).

It was followed by Group III patterns (with reactions to 83A, 84, 85 only, which are the majority; or with reactions also to other Group III phages), as the cause of 13 cases (24%). In 4 (8%) cases we had overlapping patterns of "52, 52A, 80, 81" Complex plus Group III phages (3 of which without reactions to 83A, 84, 85, however).

Strains of Group II phages were responsible for 6 cases (11%).

Therefore, according to the criterion for recognizing typically epidemic phage patterns ("at least causing 20% of the cases" [4]), there were, as we have seen, two phage-type series which fit the criterion: 1) the "52, 52A, 80, 81" Complex; and 2) the patterns of Group III, especially if 83A, 84, 85 phages reactions are present. As for the overlapping patterns of the two series they would increase a little the responsibility of either of them according to which the addition be made.

It would have been interesting to have correlated these findings to possible preventive measures in the field.

The special apparent role of phage 29 in the "52, 52A, 80, 81" Complex, and of phages 83A, 84, 85 in Group III shall be discussed in another paper [8].

Phage Patterns among Inside and Outside Hospital Acquired Staphylococcal Diseases, and Professional Staff Healthy Carriers — A significant difference (at 5% level) of phage patterns was observed in two groups of strains involved in staphylococcal diseases acquired inside and outside the Hospital. Healthy Carriers among professional staff showed and intermediate situation (Table I).

Some strains (of the "52, 52A, 80, 81") Complex; and of Group III, specially with susceptibility to phages 83A, 84, 85 only were bound to concentrate inside the hospital; Group II patterns appear more prevalent outside the hospital.

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RESUMO

Estudo de doenças estafilocócicas adquiridas em Hospital, por meio de fagotipagem realizada em Ribeirão Preto, São Paulo, Brasil

A fim de basear medidas preventivas contra doenças estafilocócicas adquiridas em hospital, em provas epidemiológicas dependentes de informações microbiológicas, um Laboratório destinado à fagotipagem de *Staphylococcus aureus* foi organizado em Ribeirão Preto, SP, Brasil.

Estudaram-se incidentes incluindo 95 casos consecutivos de doenças estafilocócicas adquiridas num Hospital-Geral-Escola, em 1966. Cinqüenta e quatro das amostras puderam ser submetidas a fagotipagem, mas sete dentre elas (13%) não foram tipáveis. Modelos bacteriofágicos do complexo "52, 52A, 80, 81" foram responsáveis em 24 (44%) casos. Modelos do Grupo III, a maioria dos quais reagindo somente com os fagos (83A, 84, 85), foram responsáveis por 13 casos (24%). Modelos combinados ("overlapping patterns") do mencionado complexo com reações aos fagos do Grupo III foram responsáveis por 4 casos (8%). O Grupo II foi responsável por 6 casos (13%).

Outrossim, diferença significativa (ao nível de 5%) na distribuição de modelos bacteriofágicos foi verificada em dois grupos de amostras provenientes dos casos de doença estafilocócica: 1) pacientes internados (54 casos, como acima referidos), e 2) pacientes de ambulatório.

Um terceiro grupo, formado por 79 portadores sãos entre pessoal profissional, dos quais 18 simultaneamente em nariz e garganta (97 cepas), mostrou posição intermediária.

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