REPORT OF THE SUBCOMMITTEE ON PHAGE-TYPING
OF STAPHYLOCOCCI OF THE INTERNATIONAL
COMMITTEE ON NOMENCLATURE OF
BACTERIA, MOSCOW, JULY 1966

The fourth meeting of the Subcommittee was held at the Gamaleya Institute, Moscow on July 22, 1966. Members present were:

Chairman, Dr. J.E. Blair, New York, N.Y., USA
Secretary, Dr. M.T. Parker, London, England

Arseni, Dr. A., Athens, Greece
Fouace, Dr. J.-M., Paris, France
Grigorova, Dr. M., Sofia, Bulgaria
Kallings, Professor L.O., Stockholm, Sweden
Kryński, Professor S., Gdańsk, Poland
Marquez, Dr. A.M., Montevideo, Uruguay
Matějovská, Dr. V., Prague, Czechoslovakia
Rische, Dr. H., Wernigerode, Germany (D.D.R.)
Rosendal, Dr. K., Copenhagen, Denmark
Rountree, Dr. P.M., Sydney, Australia
Skalova, Dr. R., Zagreb, Yugoslavia
Updyke, Dr. E.L., Atlanta, Georgia, USA (Representing Dr. P.B. Smith)
Vogelsang, Professor Th. M., Bergen, Norway
Vygodchikov, Professor G., Moscow, U.S.S.R.
Williams, Professor R.E.O., London, England

In addition, the following were present by invitation:

Mrs. E.H. Asheshov (London),
Dr. B. Bytchenko (World Health Organization),
Dr. M.P. Jevons (London), and
Dr. W. Meyer (Wernigerode).

Membership and Officers

Dr. Blair resigned from the chairmanship and from the Subcommittee. There was a general expression of gratitude for his long and distinguished service to staphylococcus phage-typing. Dr. Rountree was elected to replace him as chairman. Professor Williams resigned from the Subcommittee, and was replaced by Mrs. Asheshov. Dr. I.L. Davidson was co-opted (vide infra).

The Subcommittee's report for 1962-1966 was tabled. The Secretary reported that laboratories in an additional 14 countries were now actively engaged in Staphylococcus phage-typing and that representatives of them were therefore qualified for membership. A full list of the national representatives, who had assumed responsibility for supplying typing phages to other workers in their respective countries, or who would undertake typing for them, is appended to this Report (Appendix 1).

Liaison with the World Health Organization

In 1962 the World Health Organization recognized the Staphylococcus Reference Laboratory at the Central Public Health Laboratory, Colindale, London, as the International Centre for Staphylococcus Phage-Typing, and it has since continued to support the activities of this laboratory.

Distribution of Phages

Fresh stocks of basic-set phages and their propagating strains were issued by the International Centre to all national laboratories in November, 1965. Extra sets were sent at other times when requested.

Standard Preparations of Typing Phages

Large batches of high-titre preparations of the basic set of typing phages had been prepared at the International Centre. The contents of one ampoule were sufficient to serve both as seed phage for a propagation and as reference standard for the lytic spectrum of the locally prepared phage. Supplies would be issued to national laboratories on request at a rate of up to two ampoules of each phage per year.

Fourth Comparative Test of Phage-Typing, 1965

A set of 24 strains of Staphylococcus aureus was circulated from the International Centre to all national laboratories in 1965. Results were returned by 28 laboratories in time for analysis. They were generally encouraging and suggested that the number of laboratories obtaining results not differing materially from those of the International Centre was increasing at least as fast as the number of laboratories engaged in typing. It was felt, however, that the value of the tests would be enhanced if they were carried out more often and if the results were made available more quickly. The Secretary agreed to design a simplified form of comparative trial which could be held yearly.

Phage-typing of Staphylococci of Bovine Origin

At its last meeting, the Subcommittee asked Dr. Davidson to organize an exchange of information among veterinary bacteriologists with a view to defining a suitable set of phages for typing staphylococci from animal sources. A report from Dr. Davidson was considered at the present meeting. This described an extensive investigation carried out at the Ministry of Agriculture Central Veterinary Laboratory, Weybridge, England, of a number of the phages used for typing bovine staphylococci. As a result of this work, a provisional set of 17 phages (including 9 from the present basic typing set) had been selected for further test. Twenty-five workers had been asked to take part in a collaborative investigation of the value of these phages.

The Subcommittee approved of Dr. Davidson's activities and agreed that national representatives should supply basic-set phages to his collaborators in their respective countries; Dr. Davidson would supply the other phages. Close collaboration should be maintained between workers typing human and those typing other animal staphylococci. The phages used for typing bovine staphylococci should be fully characterized; their lytic spectra and those of the basic-set phages should be determined on a common set of indicator strains. The Subcommittee proposed the setting up of a working group to initiate this collaboration. Dr. Davidson was asked to be chairman and to invite four veterinary bacteriologists to be members; the Subcommittee would be represented by Dr. Rountree, Dr. Parker, and Dr. Meyer.

New Strains of Staphylococcus aureus causing Sepsis in Hospitals

(A) In 1960, new strains of S. aureus which were untypable with the basic set of phages or with phage 83A but which gave inhibition reactions with group III phages began to assume importance. They were observed at about the same time in the United States (Thomas, Hill, Culbertson and Altemeier 1960), Canada (Comtois and Bynoe 1963) and in Britain (Temple and Blackburn 1963). Work carried out at the International Centre suggested that the British strains had arisen from 83A staphylococci by lysogenization with one of a number of different phages, all of which blocked the reaction with phage 83A (Jevons and Parker 1964). Similar organisms are now endemic in many different countries in Europe, America and Australasia.

All of these organisms are resistant to several antibiotics, and a considerable proportion of them also have a number of unusual cultural characters, including the formation of lemon-yellow pigment on glycerol monoacetate agar, a negative Tween reaction, ability to produce β -toxin but not staphylokinase, and resistance to neomycin and bacitracin. American and Australian strains share these characters but do not appear to carry phages which lyse 83A staphylococci (Jevons, John and Parker 1966).

Seven experimental phages which might be used to characterize these new strains have been examined at the International Centre. Three of them (D, 77Ad and B5) were distributed to national laboratories in 1964, and reports on their use were presented to the meeting (Appendix 2).

(B) Kende, Balló and Ferenczi (1965) described the appearance in Hungary of a new untypable strain of S. aureus which was responsible for extensive outbreaks of skin sepsis in infants. Phage 42D was adapted to the untypable strain (see also Kende 1966). The new phage, 1380, was distributed to national laboratories early in 1966. The Subcommittee considered early reports on the use of this phage (Appendix 3), from which it appeared that the new organism had not yet spread extensively in neighbouring countries.

Constitution of the Basic Set of Typing Phages

After discussion the following decisions were made:

1. The experimental phages 77Ad and B5 should be introduced into the basic set of typing phages for the purpose of recognizing the strains described above (A).
2. These two phages, and phage 83A, should, in the future, be used for typing at RTD but not at RTD x 1000.
3. It was at present not desirable to increase the total number of phages in the set. Therefore, phages 3B and 7 should be removed, as it was generally agreed that they were the least useful of the present phages.
4. Although the experimental phage 1380 had proved useful to characterize an important strain of limited distribution, it should not be included in the basic set at the present time. National laboratories should continue to use it as an experimental phage.
5. A re-examination of phage 82 had not produced evidence for its usefulness on a worldwide scale. It should not, therefore, be included in the basic set.

6. In order to reduce confusion in published reports, new phages should be given official numbers as soon as possible. In the future, phages should be numbered serially before they are issued by the International Centre to national phage-typing laboratories for evaluation. If a phage without such an official number is mentioned in a published report on type-distribution, it should be adequately characterized and deposited with the International Centre.
7. The following official numbers were given to phages:

<u>Present designation</u>	<u>Original description</u>	<u>Official number</u>
77Ad	Parker and Jevons 1964	84
B5	Parker and Jevons 1964	85
UC18	Thomas <u>et al.</u> 1960	86
1380	Kende <u>et al.</u> 1965	87

8. The current basic set therefore includes the following phages:

Group

I	29	52	52A	79	80
II	3A	3C	55	71	
III	6	42E	47	53	54
	75	77	83A*	84*	85*
IV	42D				
Not allotted	81	187			

* used only at RTD

Technical Aspects of Phage-typing

There was some discussion of the difficulty of interpreting the results of routine typing tests with phage at RTD x 1000. Some members felt that the use of a somewhat lower concentration of phage might improve reproducibility without greatly reducing the percentage of typable cultures. Dr. Skalova agreed to form a working group to study the reproducibility of typing results with phage of different strengths. The opinion was also expressed that the constitution of the set of strains used for testing the lytic spectra of the phages might be simplified. Mrs. Asheshov agreed to look into this and to report her findings.

J.E. Blair, Chairman

M. T. Parker, Secretary

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APPENDIX 1

National Representatives for Staphylococcus Phage-Typing

- Argentina: Mrs. L. S. Saubert de Allegroni, National Institute of Microbiology, Institute Malbran, Av. Velez Sarsfield 563, Buenos Aires.
- Australia: Dr. P. M. Rountree, Fairfax Institute of Pathology, Royal Prince Alfred Hospital, Camperdown, N. S. W.
- Belgium: Dr. J. Beumer, Institut Pasteur de Brabant, rue de Remorqueur 28, Brussels 4.
- Brazil: Professor J. Oliveira de Almeida, Faculdade de Medicina de Ribeirao Preto, University of Sao Paulo, Sao Paulo.
- Britain: Mrs. E. H. Asheshov, Central Public Health Laboratory, Colindale, London, N. W. 9.
- Bulgaria: Dr. M. Grigorova, Research Institute of Epidemiology and Microbiology, Blv. VI. Zaimov 26, Sofia.

- Canada: *Mr. R. D. Comtois, Dept. of National Health and Welfare, Laboratory of Hygiene, Ottawa.
- Ceylon: Dr. Y. Brohier, Medical Research Institute, Colombo 8.
- Chile: Dr. M. Rodriguez-Leiva, Dept. Bacteriology and Immunology, Universidad Catolica de Chile, Santiago.
- China: Dr. Chang, Research Institute for Biologicals, Chendu, Szechwan.
- Czechoslovakia: Dr. V. Matějovska, Institute of Epidemiology and Microbiology, Srobarova 48, Prague X.
- Denmark: Dr. K. Rosendal, State Serum Institute, Amager Blvd. 80, Copenhagen.
- Egypt: Dr. A. El Ghoroury, Dept. Bacteriology, High Institute of Public Health, 165 Horria Avenue, Hadra, Alexandria.
- Finland: Dr. I. Rantasalo, Dept. of Hygiene, University of Helsinki, Haartmaninkatu, Helsinki 25.
- France: Dr. J.-M. Fouace, Service des Streptocoques, Institut Pasteur, 24 rue du Docteur Roux, Paris XV.
- Germany, F.R.: Professor H. Brandis, Hygiene-Institut der Universität, Kreuzberggring 57, (20b) Göttingen.
- Germany, D.D.R.: Dr. H. Rische, Bezirks-Hygiene-Institut, Burgstrasse 37, Wernigerode.
- Greece: Dr. A. Arseni, Bacteriological Laboratory, Children's Hospital 'Aghia Sophia,' Athens 609.
- Hungary: Dr. H. Milch, Staatl. f. Hygiene, Gyáli ut 2, Budapest IX.
- India: Dr. B. Ghosh Ray, Dept. Bacteriology, Maulana Azad Medical College, New Delhi.
- Iran: Dr. R. Gharagozloo, Institute of Public Health Research, Teheran University, P.O. Box 1310, Teheran.
- Israel: Dr. D. Sompolinsky, "Assaf Harofe" Hospital, Sarafand, Tel-Aviv.
- Italy: Professor V. Ortali, Istituto Superiore di Sanità, Rome.
- Japan: Professor K. Ishihara, University of Gunma, School of Medicine, Maebashi.
- Korea (South): Professor Doki Chun, Dept. Bacteriology, Kyung-pook University, Medical School, Taegu, Republic of Korea.

* It was learned with great regret that Mr. Comtois died suddenly in July, 1966.

- Mexico: Dr. L. E. Sanchez-Torres, Dept. Microbiology,
Instituto Politecnico Nacional, Escuela Nacional de
Ciencias Biologicas, Apartado Postal No. 19186,
Mexico, D. F.
- Netherlands: Dr. R. Th. Scholtens, Rijks Instituut voor de
Volksgezondheid, Sterrenbos 1, Utrecht.
- New Zealand: Dr. J. D. Manning, National Health Institute,
52 Riddiford Street, Wellington, S. I.
- Norway: Professor Th. M. Vogelsang, Gade's Institute,
Bergen.
- Poland: Professor Dr. S. Kryński, Dept. Microbiology,
Medical School of Gdańsk, Hibnera 1d, Gdańsk.
- Portugal: Dr. M. Moreira-Jacob, National Laboratory of
Veterinary Research, 701 Benfica, Lisbon 4.
- Roumania: Dr. M. Popovici, Institut "Dr. I. Cantacuzino,"
Spl. Independentei nr. 103, Raionul V. I. Lenin,
Bucaresti 35.
- South Africa: Professor A. Kipps, Dept. Pathology,
Medical School, Observatory, Cape Town.
- Spain: Dr. V. Sanchis-Bayarri Vaillant, Avenue Marques
de Sotelo 9, Valencia.
- Sweden: Dr. L. O. Kallings, State Bacteriological Labora-
tory, Box 764, Stockholm 1.
- Switzerland: Dr. H. Reber, Medizinische Universitäts-
klinik, Bürgerspital, Basel.
- Turkey: Dr. E. T. Çetin, Institute of Microbiology and
Contagious Diseases, Faculty of Medicine, Istanbul
University, Istanbul.
- Uruguay: Dr. A. M. Marquez, Facultad de Quimica y
Farmacia, Avda. Gral. Flores 2124, Montevideo.
- U. S. A.: Dr. P. Byrd Smith, Staphylococcus Unit, Bac-
teriology Section, CDC, Atlanta Georgia 30333.
- U. S. S. R.: Professor G. Vygodchikov, Gamaleya's
Institute of Microbiology, Tahukinskaja 33, Moscow.
- Viet-Nam: Dr. Vu Qui Dai, Dept. Virology, Institut
Pasteur, P. O. Box G-6, Saigon.
- Yugoslavia: Dr. R. Skalova, School of Public Health
"Andrija Stampar," University of Zagreb, Mirogojska
Cesta 4, Zagreb.

APPENDIX 2

Use of the Experimental Phages D, 77Ad and B5

The experimental phages were sent to National Laboratories in February 1964. The following tables summarize those reports which could be analyzed in a uniform manner. Most of the information refers to 1964 and early 1965, but the report from Poland covers 1965, and those from Canada and the U. A. R. extend to early 1966.

Table 1 shows the total number of cultures examined, the percentages that were typable with the basic set, and the percentage which, though untypable with the basic set, were lysed at 1 x RTD by one or more of the experimental phages. Despite differences in the manner of selection of the strains in the various centres, there is no doubt that strains lysed by these phages are now prevalent in widely separated parts of the world, though they were not found in some countries. In addition to the information given in the table, we learn that similar strains are commonly found in Australia and Denmark. Though not prevalent in New Zealand when this report was made, they are said to have appeared in 1966.

Table 2 shows the frequency with which particular patterns of lysis by the three phages were observed in otherwise untypable staphylococci, and Table 3 gives the percentages of the cultures which would have been lysed if less than three of the phages had been used.

Table 1
Frequency of untypable staphylococci which were lysed by the
experimental phages D, 77Ad and B5

	Number of cultures examined	Percentage untypable with basic phages (1 x RTD & 1000 x RTD)	Percentage untypable with basic phages but lysed at RTD by one or more of the experimental phages
Britain	85,867	24	6
[Colindale]	(15,390)	(19)	(10)
Bulgaria	1,303	17	3
Canada	6,970	30	14
Ceylon	97	37	0
Czechoslovakia	1,179	45	5
France	178	19	0
Germany, D.D.R.	287	11	0
Germany, F.R.	1,101	14	2
Holland	437	28	13
Hungary	3,883	32	0.2
Iran	308	33	6
Israel	1,153	26	5
Japan	756	11	0.1
New Zealand	3,795	5	<0.1
Norway	1,584	14	6
Poland	2,740	30	4
Roumania	2,103	45	19
Spain	242	28	6
Sweden	2,834	12	1
Switzerland	877	33	2
U.A.R.	663	25	2
U.S.A.	3,797	11	2

Table 2Frequency of patterns of lysis by the experimental phages

	<u>Number</u>	<u>Per cent</u>
D	330	4.0
77Ad	694	8.4
B5	1015	12.3
D/77Ad	596	7.3
D/B5	386	4.7
77Ad/B5	1812	22.1
D/77Ad/B5	3383	41.2
	<hr/> 8216	

Table 3

Percentage of strains lysed only by the experimental phages which
would have been typed if less than three of the phages had been used

[Includes results from countries where >10 cultures were available for analysis]

Country	Total No.	Only D	Only 77Ad	Only B5	<u>Phage</u>		
					D + 77Ad	D + B5	77Ad + B5
Britain	5501	60	76	80	86	92	97
Bulgaria	35	9	89	94	91	94	100
Canada	958	50	90	89	90	95	100
Czechoslovakia	65	0	78	75	78	75	100
Denmark	734	85	77	75	96	93	84
Holland	57	4	58	84	58	88	100
Israel	55	54	91	91	94	91	100
Norway	89	12	95	83	99	87	98
Poland	112	23	46	62	67	71	94
Roumania	405	12	98	86	99	88	99
Spain	15	60	80	40	87	73	93
Sweden	28	75	29	32	96	93	43
Switzerland	14	29	71	50	100	79	71
Iran	17	18	71	41	71	53	100

Appendix 3

Use of the experimental phage 1380

Country	Period covered	Number of cultures examined	Total	Percentage untypable with basic set	
				Lysed at RTM by phages D, 77Ad or B5	1380
Canada	Early 1966	262	19	2	1 †
Czechoslovakia	1965-66	1440	34	7	0.1
Hungary	1965-66	8332	43	0.5	16
Poland	1965-66	3567	41	8	0.6
Roumania	1964-66	4013	30	18	0.1
Yugoslavia	1965-66	1830	19	4	0
Egypt	1965-66	663	25	2	0
Sweden	Early 1966	3396	16	2	0.1
Switzerland	Early 1966	765			0
Britain	Late 1965	2140			0

† From dairy products