Serotype and sulphonamide sensitivity of meningococci isolated from 1966 to 1971

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SYNOPSIS From 1966 to 1971, 298 cultures of meningococci from clinical material (cerebrospinal fluid or blood) were examined. Eighty-nine cultures were from the Manchester area and the remainder from other parts of England or Northern Ireland. Five per cent of strains were group A, 57% group B, and 31% group C; 6% were untypable. Eighteen strains (6%) had an MIC of 6.4 µg/ml or more of sodium sulphadiazine and 10 of these (3.5%) an MIC of 50 to 100 µg/ml. The incidence of sulphonamide resistance was higher in group A strains than in group B or group C strains.

Since 1966 meningococci isolated from the blood or cerebrospinal fluid of patients with meningococcal infection have been sent to this laboratory from different parts of England and Northern Ireland for serotyping and for sulphonamide sensitivity tests.

Materials and Methods

STRAINS OF MENINGOCOCCI

All strains were oxidase-positive, Gram-negative diplococci with typical colonial morphology on blood agar at 36°C in a candle jar. Strains attacked glucose and maltose but not sucrose or lactose; occasional strains failed to attack glucose or maltose. Fermentation of sugars was carried out using B.B.L. cystine trypticase (no. 01-174) agar stabs with 1% added carbohydrate; these were incubated aerobically for at least three days. Cultures were preserved for day-to-day use on Dorset's egg slopes at 30°C, but a proportion of strains were freeze-dried.

SEROTYPING

Strains received in 1970 and 1971 were typed within a day or two of arrival. The majority of strains received before 1970 were kept for varying periods on Dorset's egg medium before they were typed. A rapid agglutination technique (Slaterus, 1962, and personal communication) was used. Cultures were subcultured from blood agar to half a plate of Bacto Mueller-Hinton agar (Difco) with 1% supplement B (Difco) and incubated in a candle jar at 36°C overnight. The following day a suspension of live

organisms was made by drawing a sterile non-absorbent cotton wool swab across the growth and emulsifying the material on the swab in 5 ml of sterile normal saline. The suspension (approximately Brown's opacity tubes 6-8) was allowed to stand for a few minutes so that any large clumps would settle to the bottom. One drop (approximately 0.02 ml) of the appropriate dilution (see below) of each typing serum was added to separate wells of a WHO plastic agglutination tray, and then 4 drops of the upper half of the suspension was added to each well; the plates were gently shaken by hand for five minutes and macroscopic agglutination was read by naked eye. Sera prepared in this laboratory against serotypes A, B, C, X, Y, and Z were used routinely. The strains used for preparing antisera were: group A Sara Branham M1027, B Sara Branham M993, C Sara Branham M1628, X Slaterus, Y Slaterus, and Z Slaterus.

Rabbits were inoculated intravenously, first with two doses of heat-killed suspensions (65°C for 30 minutes) in saline, and then with increasing doses of a suspension of live organisms in saline, usually three intravenous inoculations per week; each week a fresh ampoule of the appropriate freeze-dried strain was opened, and subcultures on Mueller-Hinton agar with 1% supplement B (Difco) used for preparing the suspension. Most sera had a titre of 1/40 against the homologous strain and were diluted 1/2 before use: sera with a titre of 1/20 were not diluted and those with a titre of 1/80 diluted 1/4. None of the sera agglutinated with the heterologous groups at the dilutions used in the test, ie, one drop of working dilution of sera and 4 drops of suspension.

SULPHONAMIDE SENSITIVITY

Tests were carried out on Bacto Mueller-Hinton agar (Difco) with sodium sulphadiazine added in varying amounts from 0.1 to 200 µg/ml. A small inoculum, 1/100-1/150 dilution of an overnight serum-broth culture (approximately 5000-10000 organisms), was applied with a phage applicator (Tarr, 1958). Plates were read by naked eye after 36 to 48 hours' incubation at 36°C in a candle jar. Inhibition was recorded when there was either no growth or fewer than 10 colonies developed.

Results

SEROTYPING

The results are shown in Table I. Of 251 strains tested, 13 (5%) were group A, 143 (57%) were group B, 79 (31%) were group C, and one each groups X and Y. Fourteen strains (6%) were untypable, but 13 of these had been isolated before 1970 and stored for a considerable time. Eighty-nine of these cultures were from the Manchester area, and the remainder from other parts of England and Northern Ireland.

Year	Group						Not Typable	Not Tested	Total
	A	B	C	X	Y	Z			
1966	1	11	5				4	10	31
1967	1	10	8				1	18	38
1968		20	10				7	9	46
1969	1	26	10				1	10	48
1970	6	40	24						70
1971 (Jan-Sept)	4	36	22	1	1		1		65
Total	13	143	79	1	1		14	47	298

Table I Serotypes of 298 strains of meningococci from clinical infections

SULPHONAMIDE SENSITIVITY

The results of testing 282 strains are shown in Table II. These comprised 13 group A, 140 group B, 75 group C, 13 untypable, and one group Y strain, as well as 40 strains that had not been serotyped. Eighteen strains (6%) had an MIC of 6.4 µg/ml or more. Eight strains had a low level of resistance (MIC 6.4-10 µg/ml) and 10 strains had higher levels (MIC 50-100 µg/ml). Further details of the resistant strains are shown in Table III. A higher proportion of group A strains (31%) were resistant

Serotype	Number of Strains Inhibited by Sodium Sulphadiazine (µg/ml)			Total No. of Strains
	6.4 & 10	50	100	
Group A	2	2		4
Group B	3	3	1	7
Group C	2	3	1	6
Not typable	1			1

Table III Serotype of sodium sulphadiazine-resistant strains of meningococci

compared with 5% of group B and 8% of group C strains.

Discussion

Before 1970 a method of typing using heated suspensions and agglutination at 50°C (Memorandum No. 4, Public Health Laboratory Service, 1961) had been used (Abbott, Adams, and Collins, 1970) and by this method just over half of strains from clinical material were untypable. Since 1970 the rapid agglutination test described by Slaterus (1962) was adopted and the serotyping reported here was by this method. A comparison of results by the two methods showed little correlation. The rapid test is simple, yields consistent results correlating well with the epidemiological information, and the vast majority of strains from clinical specimens are typable. Group B (57%) and group C (31%) are the commonest types, while group A strains (5%) are relatively uncommon. These results are comparable with those found in strains isolated in Holland (Severin, Ruys, Bijkerk, and Butter, 1969) and from the USA (Ivler, Leedom, Mathies, Fremont, Thrupp, Portnoy, and Wehrle, 1966; Wiggins and Schubert, 1967), although the proportion of group A strains in the USA is even less. Recently group C strains appear to have overtaken group B strains as the commonest type in the USA (Wiggins, McLaughlin, Bickham, Jones, and Balows, 1970).

By the technique described, a number of sulphonamide-resistant strains were found amongst 282 cultures tested. Eighteen strains (6%) had an MIC of 6.40 µg/ml or more, and 10 required 50-100 µg/ml to inhibit growth. The results of testing 88 of these strains were reported previously (Abbott *et al.*, 1970). Fallon (1971) has also reported the presence of sulphonamide-resistant strains in Scotland. It is

Number of Strains Inhibited by Sodium Sulphadiazine (µg/ml)											Total Number of Strains
0.1	0.2	0.4	0.8	1.6	3.2	6.4	10	50	100	Not Tested	
5	21	82	84	60	12	7	1	8	2	16	298

Table II Sensitivity to sodium sulphadiazine of 298 strains of meningococci

evident, therefore, that sulphonamides alone can no longer be relied upon for the treatment of clinical disease. Although only a small proportion of strains (6%) were resistant to sulphonamides the incidence of resistance amongst group A strains was higher (31%). The overall situation in this country is different from the USA where Wiggins *et al* (1970) reported that nearly two out of three of the strains from clinical cases submitted to the CDC during 1970 were resistant to 10 μ g/ml or more of sulphadiazine.

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