Epidemiol. Infect. (1992), **108**, 449–455
Printed in Great Britain

Antibiotic sensitivities of *Neisseria meningitidis* isolates from patients and carriers in Greece

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(*Accepted 10 November 1991*)

SUMMARY

Usage of antibiotics in southern Europe is less well regulated than in northern countries. The proportion (48%) of meningococci in Spain insensitive to penicillin (MIC $\geqslant$ 0·1 mg/l) prompted this investigation of antibiotic sensitivities of isolates from Greek patients with meningitis (31) and carriers (47 school-children and 472 recruits). The agar dilution method was used to determine MIC to penicillin G (PN), sulphamethoxazole (SU), rifampicin (RF), cefaclor (CF) and ciprofloxacin (CP).

The proportion of isolates insensitive to PN was 48% for isolates from patients, 19% from school-children and 36·6% from recruits. Resistance to SU (MIC $\geqslant$ 16 mg/l) was found in 16% of those from patients, 10·6% from children and 40% from recruits. None of the isolates from patients was resistant to RF ($\geqslant$ 1 mg/l) but 6% of those from carriers were. Resistance to CF ($\geqslant$ 4 mg/l) was found in 9·2% of patient isolates, 6·4% from children and 23·7% from recruits. All isolates except one were sensitive to CP (MIC range $<$ 0·0015–0·125 mg/l).

Resistances to PN, SU and RF were analysed by serogroup, serotype and subtype of the bacteria. The proportion of resistant isolates showed some variation between different areas of Greece, but it was not statistically significant.

INTRODUCTION

As part of the surveys of isolates of meningococci from patients and carriers in Greece, the antibiotic sensitivities of these bacteria to some common antibiotics were determined. There are reports of *Neisseria meningitidis* with reduced sensitivity to penicillin isolated from patients in Spain [1, 2], South Africa [3], and the United Kingdom [4, 5]. A small proportion of these penicillin-insensitive isolates has also been found among those obtained from carriers in the United Kingdom [4]. In view of the increasing prevalence of penicillin-insensitive isolates

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in Spain and the similar antibiotic policies of both Spain and Greece, it was predicted that there might be significant numbers of Greek isolates with decreased sensitivity to penicillin and other antibiotics.

The objectives of the study were: (1) to compare levels of sensitivities for isolates obtained from patients with those obtained from carriers; (2) to determine if the proportion of penicillin-insensitive isolates is comparable to that reported for Spain; (3) to assess the distribution of resistant isolates in the different regions of Greece; (4) to determine if antibiotic resistances are associated with particular serogroups, serotypes or subtypes.

ISOLATES

Meningitis cases

Thirty-one isolates from children aged 1 month to 11 years were provided by the microbiology laboratory of the 'Aglia Kyriakou' Paediatric Hospital. All cases were sporadic and occurred during 1989–91.

Carriers

Four hundred and seventy-two isolates were obtained during 1990–1 from healthy recruits aged 16–31 years [6] and a further 47 from children aged 6–18 years who did not have meningococcal disease.

METHODS

Antibiotic sensitivities to penicillin (PN), erythromycin (ER), tetracycline (TC), sulphamethoxazole (SU), rifampicin (RF), cefaclor (CF) and ciprofloxacin (CP) were determined by the agar dilution method. A range of twofold dilutions from 256 to 0·015 mg/l of each antibiotic in Mueller Hinton medium was examined. The breakpoints used for penicillin ($\geqslant$ 0·125 mg/l), tetracycline ($\geqslant$ 1 mg/l), erythromycin ($\geqslant$ 0·5 mg/l), rifampicin ($\geqslant$ mg/l), ciprofloxacin ($\geqslant$ 1 mg/l) and the cephalosporin cefaclor ($\geqslant$ 4 mg/l) were based on those recommended by the working party of the British Society for Antimicrobial Chemotherapy for *Branhamella catarrhalis* and *Haemophilus influenzae* [7]. The break point of $\geqslant$ 10 mg/l for sulphamethoxazole (SU) was that used by the Neisseria Reference Laboratory (Scotland) [Dr R. J. Fallon, personal communication]. Beta-lactamase production was assessed during the rapid carbohydrate fermentation test for identification of each isolate [8].

Serogroup was determined by slide agglutination with commercially available antisera (Wellcome Diagnostics); serotype and subtype were determined by whole cell enzyme-linked immunosorbent assays with monoclonal antibodies from Dr J. T. Poolman (RIVM Bilthoven, The Netherlands) [9].

RESULTS

Table 1 compares the proportion of resistant isolates among the three groups examined. Among 31 isolates obtained from children with meningococcal disease, none was resistant to rifampicin or ciprofloxacin. Nearly half the isolates were penicillin-insensitive (48·3%) and all grew on 1 or 2 mg/l tetracycline.

Among the 47 isolates from school-children the pattern of resistance to

Table 1. *Proportion of antibiotic-resistant isolates among patients and carriers*

	Percent resistant		
		Carriers	
	Patients ($n = 31$)	Children ($n = 47$)	Recruits ($n = 472$)
Penicillin G	48·3	19·1	36·7
Sulphamethoxazole	16·1	10·6	35·6
Rifampicin	—	6·4	7
Tetracycline	100	6·4	91
Erythromycin	25·8	61·7	82·2
Cefaclor	9·7	6·4	23·7
Ciprofloxacin	—	—	0·2

ciprofloxacin, cefaclor and sulphamethoxazole was similar to that of isolates from patients. The proportion resistant to erythromycin (61·7%) was over twice that among isolates from patients (25·8%) ($\chi^2 = 8{\cdot}27$, df = 1, $P = < 0{\cdot}005$); and there were some rifampicin resistant strains (6·4%). Compared with the isolates from patients, the proportion resistant to tetracycline was much less (6·4%) ($\chi^2 = 62{\cdot}83$, df = 1, $P = < 0{\cdot}0005$) as was the proportion with decreased sensitivity to penicillin (19·1%) ($\chi^2 = 7{\cdot}61$, df = 1, $P = < 0{\cdot}01$).

The proportions of 472 isolates from recruits resistant to tetracycline or penicillin were similar to those of isolates from patients. The proportions of isolates from recruits resistant to erythromycin or to rifampicin were similar to that for isolates from children who were carriers. Compared with the other two groups, there was a higher proportion of isolates from recruits resistant to sulphamethoxazole and cefaclor. There were 112 isolates (23·7%) with MIC $\geqslant$ 4 mg/l cefaclor and 32 isolates (6·8%) with MIC $\geqslant$ 8 mg/l. Of the latter 32 isolates, 27 (84%) had decreased sensitivity to penicillin. There was one isolate with MIC $\geqslant$ 1 mg/l ciprofloxacin. None of the 550 isolates from patients and carriers produced beta-lactamase.

Geographical distribution of resistant isolates

In the two major population centres, Eastern Macedonia and Athens, the proportions of penicillin-insensitive isolates were 33 and 40% respectively. The highest proportions of such isolates were found in Thrace (50%), Crete (52%) and the Aegean and Ionian Islands (50%). The lowest proportion was obtained from recruits who lived abroad but who had returned to Greece for their compulsory military service (9%). Analysis of the serogroups, serotypes, subtypes and sensitivities to other antibiotics indicate that the isolates within a particular geographical area with decreased sensitivity to penicillin are not related (Table 2).

Analysis by antigenic phenotypes

Meningitis strains

Among the isolates from patients with meningitis, 50% of group B, 37·5% of group C and 60% of the non-groupables were penicillin-insensitive. Sulphonamide resistance was found in 17% of the group B isolates and 25% of group C but in

Table 2. *Proportion of antibiotic-resistant isolates obtained from recruits from different regions of Greece*

Region	Number tested	Percent resistant PN	SU	RF	CP
Thrace	14	50	79	7	—
W. Macedonia	38	26	42	5	—
E. Macedonia	96	33	35	7	—
Epirus	23	48	30	4	—
Thessaly	46	34	48	6	—
Central Greece	32	32	44	9	—
Athens	127	40	39	7	0·8
Peloponnese	40	35	30	10	—
Crete	31	52	39	3	—
Aegean Islands	10	50	70	10	—
Ionian Islands	2	50	—	50	—
Foreign	11	9	22	—	—

Table 3. *Antibiotic resistances of serogroups*

Serogroup	No.	Percent resistant PN	SU	RF
A	17	41	41	12
B	114	35	37	5
C	19	42	42	10·5
W135	2	0	50	0
Y	8	62·5	25	0
Z	4	25	0	0
NG	300	36	40	7

none of the non-groupable isolates. Of the 9 2b serotype isolates, 7 (78%) were penicillin-insensitive as were 8/18 (44%) of the non-typables. The 4 isolates expressing other serotypes (2a, 14 and 15) were sensitive to penicillin and to sulphamethoxazole. Resistance to sulphamethoxazole was found for only 2/9 serotype 2b isolates and 3/18 non-typable isolates. Of the 9 isolates expressing the P1.10 subtype, 7 (78%) were penicillin-insensitive though only 1 was resistant to sulphamethoxazole. The second most common subtype was P1.9. Only 1 of the 5 P1.9 isolates was penicillin-insensitive, but 2 were sulphonamide resistant.

Carrier strains

There were sufficient numbers of isolates from the recruits for analysis of antibiotic resistances by serogroup, serotype and subtype (Tables 3–5). Among the serogroups, the highest proportion of penicillin-insensitive isolates was found for group Y (62·5%) while rifampicin resistance was greatest in groups A (12%) and C (10·5%) (Table 3).

Penicillin-insensitivity was highest among serotypes 2a and 15 and lowest in type 4, which, however, had the highest proportion of strains resistant to sulphamethoxazole. Rifampicin resistance was observed most frequently among serotypes 4 and 15 (Table 4).

Table 4. *Antibiotic resistances among serotypes*

Serotype	No.	Percent resistant PN	SU	RF
1	15	27	20	7
2a	7	57	43	0
2b	22	27	32	4·5
4	9	11	56	11
14	66	42	28	9
15	16	56	19	12·5

Table 5. *Antibiotic resistances among subtypes*

Serogroup	No.	Percent resistant PN	SU	RF
1	41	39	34	2·5
2	72	43	22	15·5
4	7	43	29	0
6	28	32	68	0
7	30	43	63	13
9	45	27	38	2·4
10	29	45	28	14
12	8	25	37·5	0
14	8	62·5	25	0
15	16	37·5	19	0
16	13	38	70	23

No particular subtype antigen was associated with an unusual degree of antibiotic resistance (Table 5).

DISCUSSION

Although sulphonamide resistance has been associated with strains causing outbreaks of meningococcal disease in northern Europe [10, 11], only 16% of the isolates from Greek patients had MIC > 10 mg/l to sulphamethoxazole; however, nearly half had reduced sensitivity to penicillin and all grew in the presence of 1 or 2 mgm/l tetracycline.

In contrast, while the proportions of penicillin-insensitive and of tetracycline-resistant isolates from carrier children were both significantly lower, the proportion of erythromycin isolates was significantly increased.

Among isolates from the recruits, the proportion of penicillin-insensitive strains was significantly greater than that from children who were carriers ($\chi^2 = 5{\cdot}126$, df = 1, $P < 0{\cdot}025$) but not compared with those from children with disease. The proportion of sulphonamide-resistant isolates among those from recruits (35·6%) was over twice that of the other two groups, carriers (10·6%) ($\chi^2 = 10{\cdot}882$, df = 1, $P < 0{\cdot}005$) or patients (16%) ($\chi^2 = 4{\cdot}06$, df = 1, $P < 0{\cdot}05$) (Table 1).

Although penicillinase-producing isolates of *Neisseria gonorrhoeae* are not uncommon in Greece (18%) [12], none of the 550 meningococcal isolates tested produced beta-lactamase.

https://doi.org/10.1017/S0950268800049955 Published online by Cambridge University Press

The levels of resistance to erythromycin among isolates from carriers might reflect longer exposure to the antibiotic as the mean ages of the carriers were higher, school-children (12·8 years) and recruits (19·7 years) compared with a mean age of 2·7 years for patients with meningitis. Only one isolate had MIC $\geqslant$ 1 mg/l to ciprofloxacin. Since 6–7% of isolates from carriers were resistant to rifampicin, ciprofloxacin might be considered for circumstances when chemoprophylaxis would be appropriate as it has been found to eradicate pharyngeal carriage of meningococci [13].

Sutcliffe and colleagues [4] stated that in view of the extensive use of penicillin in the community in Britain, emergence of penicillin-insensitive meningococci is not unexpected. In Britain, the proportion of isolates from patients and carriers with MIC > 0·16 mg/l was about 3% [4]. In Spain in 1986, only 5% of isolates examined had MIC $\geqslant$ 0·1 mg/l; however, by 1990, this figure had risen to 46% of isolates from patients. The antibiotic policies of Spain and Greece are similar and the proportion of penicillin-insensitive isolates from Greek children with meningococcal disease (48·3%) was similar to that reported for Spain. In Britain, the proportion of penicillin-insensitive isolates from carriers was slightly less than 3% [4], much lower than the proportion of penicillin-insensitive strains from carriers in this survey, recruits (36%) or school-children (19·1%).

Differences in MIC values to tetracycline between isolates from patients and isolates from children who were carriers was not expected. Although high level tetracycline resistance mediated by a plasmid carrying the *tetM* determinant has been found in meningococci [14] none of the isolates had MIC > 8 and the 25·2 megadalton plasmid was not observed in any of the strains examined so far [unpublished observations].

The differences in the proportions of penicillin-insensitive isolates in the different regions of Greece is under investigation (Table 2). That there were fewer such isolates among recruits who lived outside Greece (mainly in Germany) might reflect differences in antibiotic policies referred to earlier.

Although 61% of the British isolates which were penicillin-insensitive were also sulphonamide-resistant, none were resistant to rifampicin, aminoglycosides, erythromycin, tetracycline or chloramphenicol [4]. Only 37% of the penicillin-insensitive isolates in this study were sulphonamide-resistant; however, additional resistances were often present: rifampicin (8%); erythromycin (33%); cefaclor (34%); tetracycline (96%).

While none of the penicillin-insensitive strains in Britain was serogroup A [4], 41% of the Greek group A isolates had reduced sensitivity to penicillin. The highest proportion of penicillin-insensitive isolates was among the 8 group Y isolates (62·5%). Analysis by areas of residence of the recruits, serotype, subtype and sensitivities to other antibiotics suggest these group Y isolates are not related. Although serogroup B was the most common among patient isolates, the greatest proportion of isolates resistant to rifampicin was among those of groups A and C.

The number of serotypable isolates was small, and larger numbers need to be examined to determine if the associations noted are significant (Table 4). Most of the penicillin-insensitive isolates expressing subtype P1.14 do not appear to be a single strain as assessed by the serogroup, serotype, sensitivities to other antibiotics and the area of residence of the recruits from whom the bacteria were

isolated. Although the sulphonamide and rifampicin resistances were highest among subtype P1.16, the two resistances were not found together in the P1.16 strains. The higher rate of rifampicin resistance among isolates expressing P1.10, the subtype found most frequently among meningococci isolated from patients, and among isolates expressing P1.2, the most common subtype among carriers, needs to be further investigated.

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