Epidem. Inf. (1987), **99**, 463–469
Printed in Great Britain

Isolation of *Neisseria meningitidis* strains with increase of penicillin minimal inhibitory concentrations

By J. A. SÁEZ-NIETO, D. FONTANALS, J. GARCIA DE JALON, V. MARTINEZ DE ARTOLA, P. PEÑA, M. A. MORERA, R. VERDAGUER, I. SANFELIU, C. BELIO-BLASCO, J. L. PEREZ-SAENZ and J. CASAL

Centro Nacional de Microbiología, Virologia e Immunologia Sanitarias, Majadahonda, Madrid; Clinica Infantil Nen Jesus, Sabadell, Barcelona; Hospital Virgen del Camino, Pamplona; Ciudad Sanitaria La Paz, Madrid; Hospital de Sant Llátzer, Terrassa, Barcelona; Hospital de Bellvitge, Hospitalet, Barcelona; Hospital de San Juan de Dios, Barcelona; Hospital de San Millan, Logroño; Hospital San Jaime y Santa Magdalena, Mataró, Barcelona, Spain.

(*Accepted 10 March 1987*)

SUMMARY

We report the isolation and characterization of ten strains showing an increase in the minimal inhibitory concentrations to penicillin (MICs > 0·1 μg/ml), and describe the epidemiological, clinical and microbiological features.

The susceptibility of 3432 meningococcal strains isolated from patients in the recent epidemic wave (1978–86) in Spain, to several antimicrobial agents used in the treatment and chemoprophylaxis of meningococcal infection has been tested. Most were resistant to sulphadiazine but sensitive to other antibiotics.

The possible existence of a new pattern of behaviour of meningococcal to penicillin is discussed.

INTRODUCTION

Neisseria meningitidis has been extremely susceptible to penicillin, and use of this antimicrobial agent has markedly reduced the mortality of meningococcal infections. However, the morbidity of this disease has not shown a similar decrease, as can be seen from the incidence of meningococcal meningitis in many countries including Spain, during the last 8 years (1979–85). In that period, Spain has suffered the biggest epidemic of the present century with 35870 reported cases and rates of 17·6 per 100000 inhabitants in 1979 (peak of epidemic wave) and 8·7 in 1984 (Sáez-Nieto, 1985).

During this period, The National Reference Laboratory for meningococci has been performing microbial surveillance of the isolated strains using epidemiological markers (serogrouping, serotyping, and electrophoretic patterns) and by measuring their susceptibility to antimicrobial agents, including those commonly used in the treatment and the chemoprophylaxis of meningococcal infections (Sáez-Nieto, Vazquez & Casal, 1983).

In contrast to the normal marked susceptibility of meningococcal isolates to penicillin (MICs < 0·05 μg/ml) we describe here the appearance of ten strains with moderate penicillin resistance, one of them isolated in October 1985 and the other nine in the first 6 months of 1986, a fact that may alter our approach to the presumed uniformity of the response of meningococci to penicillin.

MATERIAL AND METHODS

Strains

A total of 3264 strains of *N. meningitidis* isolated from patients between 1978 and 1985 and 162 strains isolated in 1986 (January–June) was studied. The sources included cerebrospinal fluid (CSF) and blood. These strains were sent to the National Reference Laboratory from 64 hospital laboratories in Spain.

Prior to the study, we confirmed the identity of strains by means of the usual tests in our laboratory: oxidase, aminopeptidase activity, sugar degradation test, and epidemiological markers (serogrouping and serotyping) according to the methods previously described (Sáez-Nieto *et al.* 1981*a*, *b*; 1982).

Antimicrobial susceptibility

Sulphadiazine sensitivity was tested on plates containing Mueller–Hinton Agar (Difco) by adding 1, 5, 10, 25, 50 and 100 mg/l to the medium. Meningococcal suspensions were prepared in phosphate-buffered saline pH 6·9 from overnight cultures on blood agar plates. Each of these suspensions was adjusted to a concentration of 3×10^5 c.f.u./ml. To verify this concentration, total counts were performed on each set of inocula by subculture.

The inoculum was placed on the agar plates using an automatic multi-inoculator (Microtiter AM80); incubation was at 37 °C in a humid atmosphere for 18 h.

A screening concentration of the following antibiotics: penicillin (0·1 mg/l), ampicillin (0·1 mg/l), rifampicin (0·1 mg/l), chloramphenicol (0·8 mg/l) and spiramycin (3·2 mg/l) was also studied. Strains showing MICs > 0·1 μg/ml, were tested at higher penicillin and ampicillin concentrations in twofold dilutions ranging 0·1–6·4 μg/ml.

Beta-lactamase activity was studied by the acidimetric method, using commercially available beta-lactamase detection papers (Oxid).

Disk diffusion test

Strains with penicillin MICs > 0·1 μg/ml (10 strains) and 20 strains with MICs lower than these concentrations were studied by means of a disk diffusion test against the following antibiotics: penicillin (10 IU), oxacillin (1 μg), erythromycin (15 μg), tetracycline (30 μg), chloramphenicol (30 μg), trimethoprim–sulphamethoxazole (25 μg), cefuroxime (30 μg), cefoxitin (30 μg), cefotaxime (30 μg), cephalothin (30 μg) and cefoperazone (75 μg).

The suspension previously described was spread onto the agar plates by inoculating in four directions with a sterile cotton swab soaked into the inoculum. Mueller–Hinton Agar (Difco) was supplemented with 5% sheep blood and incubated at 37 °C for 18–24 h in humid atmosphere.

Table 1. *Susceptibility to antimicrobial agents of 3264 strains of meningococci isolated from patients with meningococcal infection between 1978 and 1985 in Spain*

Drug	Screening concentration (μg/ml)	Sensitive	Moderately resistant	Resistant	Total
Penicillin	0·1	3263	1*	0	3264
Ampicillin	0·1	3193	71†	0	3264
Rifampicin	0·1	3083	171‡	10‡	3264
Spiramycin	3·2	1500	0	0	1500
Chloramphenicol	0·8	1500	0	0	1500
Sulphadiazine	—§	58	435	2771	3264

* See Table 3.
† All the strains are MICs ⩽ 0·4 μg/ml.
‡ Only 10 strains showed MICs > 3·2 μg/ml.
§ MICs of sulphadiazine: ⩽ 1 (sensitive); 5–10 (moderately resistant); ⩾ 25 (resistant) (μg/ml).

Table 2. *Susceptibility to antimicrobial agents of 168 strains of meningococci isolated from patients with meningococcal infection in 1986 (January–June) in Spain*

Drug	Screening concentration (μg/ml)	Sensitive	Moderately resistant	Resistant	Total
Penicillin	0·1	159	9*	0	168
Ampicillin	0·1	152	16†	0	168
Rifampicin	0·1	158	10‡	0	168
Spiramycin	3·2	168	0	0	168
Chloramphenicol	0·8	168	0	0	168
sulphadiazine	—§	3	20	145	168

* See Table 3.
† All the strains are MICs ⩽ 0·4 μg/ml.
‡ All the strains are MICs ⩽ 0·8 μg/ml.
§ See Table 1.

RESULTS

Tables 1 and 2 show the results of the antibiotic susceptibility surveillance carried out during the period 1978–85 and 1986 (January–June). The majority of strains (85%) were resistant to sulphadiazine but none was resistant to spiramycin and only ten strains resistant to rifampicin with MICs higher than 3·2 μg/ml, were observed. None of these latter strains was isolated during the first 6 months of 1986.

All strains were susceptible to chloramphenicol. However 2·2% presented a moderate resistance to ampicillin (MICs between 0·1 and 0·4 μg/ml), a figure which grew to 10% in 1986.

Only one strain was found with an MIC > 0·1 μg/ml to penicillin between 1978 and 1985 (isolated in October 1985), however nine of these moderately resistant

https://doi.org/10.1017/S0950268800067960 Published online by Cambridge University Press

Table 3. *Characteristics of cases of meningococcal infection with strains moderately resistant to penicillin (MIC > 0·1 μg/ml). Clinical and epidemiological data*

Case	Location	Date	Age	Sex	Clinical syndrome	Treatment
1	Madrid	Oct. 1985	8 m*	♂	Sepsis and meningitis	Penicillin G
2	Barcelona	Jan. 1986	3 y	♂	Sepsis	Penicillin G
3†	Barcelona	Jan. 1986	73 y	♀	Fulminant sepsis	Penicillin G
4	Pamplona	Feb. 1986	8 m	♂	Sepsis	Ampicillin
5	Pamplona	Feb. 1986	2 y	♂	Sepsis	Ampicillin
6†	Barcelona	Mar. 1986	1y	♂	Fulminant sepsis	Penicillin G
7	Logroño	Mar. 1986	6 y	♂	Meningitis	Penicillin G
8	Barcelona	Mar. 1986	1 y	♀	Sepsis	Penicillin G
9	Barcelona	Mar. 1986	2 y	♀	Sepsis	Cefotaxime
10	Barcelona	May 1986	7 y	♀	Meningitis	Penicillin G

* m, months; y, years.
† Cases 3 and 6 died.

Table 4. *Characteristics of cases of meningococcal infection with strains moderately resistant to penicillin. Microbiological data*

Case	Source	Serogroup	Serotype	Antimicrobial MICs (μg/ml)					
				Sul*	Pen†	Amp	Rif	Clo	Spi
1	Blood	B	NT‡	50	0·2	0·2	⩽ 0·1	⩽ 0·8	⩽ 3·2
2	Spinal fluid	B	12	> 100	0·2	0·4	⩽ 0·1	⩽ 0·8	⩽ 3·2
3	Spinal fluid	B	NT	50	0·2	0·2	⩽ 0·1	⩽ 0·8	⩽ 3·2
4	Blood	B	NT	50	0·2	0·2	⩽ 0·1	⩽ 0·8	⩽ 3·2
5	Spinal fluid	B	12	25	0·2	0·2	⩽ 0·1	⩽ 0·8	⩽ 3·2
6	Spinal fluid	B	1	25	0·2	0·2	⩽ 0·1	⩽ 0·8	⩽ 3·2
7	Spinal fluid	C	NT	50	0·2	0·4	⩽ 0·1	⩽ 0·8	⩽ 3·2
8	Blood	C	2	50	0·2	0·2	⩽ 0·1	⩽ 0·8	⩽ 3·2
9	Blood	C	2	50	0·4	0·4	⩽ 0·1	⩽ 0·8	⩽ 3·2
10	Spinal fluid	C	NT	50	0·2	0·2	⩽ 0·1	⩽ 0·8	⩽ 3·2

* Sul, sulphadiazine; Pen, penicillin; Amp, ampicillin; Rif, rifampicin; Clo, Chloramphenicol; Spi, spiramycin.
† All the strains were beta-lactamase non-producers.
‡ Non-typable strains.

strains were detected in the first 6 months of 1986 (5·3% of the 168 strains studied during this period).

Clinical, epidemiological and laboratory data relating to these strains are shown in Tables 3 and 4.

The ten strains that were moderately resistant to penicillin were isolated in four different areas of Spain: 6 in Barcelona and neighbouring villages (North-east), 2 in Pamplona (North), 1 in Logroño (North) and 1 in Madrid. The patients varied in age from 8 months to 73 years. Six were males and four females.

In the period July–December 1986 four new strains with MICs > 0·1 μg/ml have been isolated from patients in the area of Barcelona (data not registered in the Tables). Five of the original cases presented as sepsis without meningitis, two as meningitis, two cases as fulminant meningococcemia and one as sepsis plus meningitis. Six patients were treated with penicillin G, two of them (those with

https://doi.org/10.1017/S0950268800067960 Published online by Cambridge University Press

Table 5. *Disk diffusion test for meningococcal strains moderately resistant and sensitive to penicillin*

Antibiotic	Disk concentrations	Halo range (mm)		Media (mm)	
		Sensitive*	Moderately† resistant	Sensitive	Moderately resistant
Penicillin G	10 IU	30–40	21–26	33·6	23·7
Oxacillin	1 μg	10–15	0	12·9	0
Erytromycin	15 μg	26–32	23–30	28·6	25·9
Tetracyclin	30 μg	25–30	25–28	28·3	26·2
Chloramphenicol	30 μg	29–36	28–34	31·9	31·3
S×T‡	25 μg	0–22	0–12	6·5	1·2
Cefuroxime	30 μg	34–43	23–33	38·5	26·7
Cefoxitin	30 μg	29–37	28–33	33·1	20·3
Cefotaxime	30 μg	36–48	32–40	41·5	36·0
Cefalothin	30 μg	28–38	22–29	33·5	25·0
Cefoperazone	75 μg	32–42	27–39	36·4	32·1

* Twenty strains.
† Ten strains.
‡ S×T, sulphamethoxazole/trimethoprim.

fulminant meningococcaemia) died, while the resolution was satisfactory in the rest (two patients were treated with ampicillin and one with cefotaxime). Case 8 was treated with cefotaxime for the first 24 h and thereafter with penicillin G.

Six strains belonged to serogroup B and four to serogroup C. Five were non-typable (NT) by immunodiffusion, two were serotype 12, one serotype 1, and two (both serogroup C) serotype 2.

The ten strains were resistant to sulphadiazine and susceptible to spiramycin, rifampicin and chloramphenicol. The MICs to penicillin and ampicillin are shown in Table 4. Only one showed a penicillin MIC of 0·4 μg/ml and the rest 0·2 μg/ml. MICs to ampicillin were: 0·4 μg/ml (3 strains) and 0·2 μg/ml (7 strains).

A clear difference in zone diameter could be seen between the moderately resistant and susceptible strains (Table 5) when tested by the disk diffusion susceptibility test, against penicillin, oxacillin and some cefalosporins.

None of these strains was beta-lactamase producing.

DISCUSSION

Meningococcal strains isolated in Spain during the last 7 years were highly resistant to sulphadiazine but susceptible to other antibiotics (rifampicin, spiramycin, ampicillin, chloramphenicol) and especially, to penicillin, the drug of choice for the treatment of infections caused by *N. meninigitidis*. However, in this paper, we describe ten strains of meningococci isolated from patients in recent months that showed decreased susceptibility to penicillin with MICs 0·2–0·4 μg/ml. According to the criteria proposed by several authors for other meningeal pathogens, these strains could be considered as moderately resistant strains (Jacobs *et al.* 1978; Ward, 1981). The emergence of such strains in a short and recent period suggest the possibility that a change in the behaviour of *N. meningitidis* in relation to its susceptibility to penicillin is taking place.

https://doi.org/10.1017/S0950268800067960 Published online by Cambridge University Press

Meningococcal resistance to penicillin has rarely been reported. In 1970, Devine & Hagerman described one strain isolated from a carrier with a MIC of penicillin of 0·25 μg/ml; previously Eickhoff & Finland (1965) reported some strains with MICs between 0·1 and 0·2 μg/ml. The same authors in 1971 also described some strains with similar MICs. Scribner *et al.* (1982) described a few strains with penicillin MICs of 0·25 μg/ml. Also, Dillon, Pauze & Yeung (1983) described the isolation of a beta-lactamase producing *N. meningitidis* strain. Contoyiannis & Adamopoulos (1974) reported several cases of meningococcal meningitis that did not respond to standard penicillin G treatment. Similar observations have been made in Spain (Dominguez & Mingella, 1985; Mancebo *et al.* 1985). However, MICs of penicillin were not reported by the last authors cited.

It is too soon to predict if the strains reported here, which show decreased susceptibility to penicillin, could represent a potential risk in the treatment of meningococcal infections. Nevertheless, it must be remembered that standard penicillin G therapy produces peaks in the CSF of 0·8 μg/ml, close to the MIC reached by one of our strains (Hieber & Nelson, 1977).

In our study, all the patients responded satisfactorily to treatment with penicillin G or other beta-lactam antibiotics, except for the two fatal cases of fulminant meningococcemia. These good responses give us confidence in contrast to the therapeutic problem posed by the emergence of pneumococcal strains showing high and moderate resistance to penicillin (Jacobs *et al.* 1978).

In Spain, the problem could be more acute, in that increasing resistance in other meningeal pathogens such as pneumococci and *Haemophilus influenzae* has been widely described (Casal, 1982; Liñares *et al.* 1983; Campos, Garcia-Tornel & Sanfeliú, 1984). However, the present data alerts us to perform susceptibility testing to penicillin and alternate drugs for meningococcal isolates.

The 10 IU penicillin disk clearly discriminates between susceptible and moderately resistant strains (zone diameter < 26 mm) and could be useful in the screening for potential resistance to penicillin.

The excellent technical assistance of Carmen Marcos is gratefully acknowledged. Also thanks to Dra. Cecilia Martín-Boungón for useful suggestions.

REFERENCES

CAMPOS, J., GARCIA-TORNEL, S. & SANFELIU, I. (1984). Susceptibility studies of multiply resistant *Haemophilus influenzae* isolated form pediatric patients and contacts. *Antimicrobial Agents and Chemotherapy* **25**, 706–709.

CASAL, J. (1982). Antimicrobial susceptibility of *Streptococcus pneumoniae*: Serotype distribution of penicillin-resistant strains in Spain. *Antimicrobial Agent and Chemotherapy* **22**, 222–225.

CONTOYIANNIS, P. & ADAMOPOULOS, D. A. (1974). Penicillin-resistant *Neisseria meningitidis*. *Lancet* i, 462.

DEVINE, L. F. & HAGERMAN, C. R. (1970). Spectra of susceptibility of *Neisseria meningitidis* to antimicrobial agents in vitro. *Applied Microbiology* **19**, 329–334.

DILLON, J. R., PAUZE, M. & YEUNG, K. H. (1983). Spread of penicillinase-producing and transfer plasmids from gonococcus to *Neisseria meningitidis*. *Lancet* i, 779–781.

DOMINGUEZ, P. & MINGUELLA, I. (1985). Existencia de cepas de meningococo resistentes a penicilina. *Medicina Clínica* **85**, 517–518.

EICKHOFF, T. C. (1971). *In vitro* and *In vivo* studies of resistance to rifampin in meningococci. *Journal of Infectious Diseases* **123**, 414–420.

EICKHOFF, T. C. & FINLAND, M. (1965). Changing susceptibility of meningococci to antimicrobial agents. *New England Journal of Medicine* **272**, 395–398.

HIEBER, J. P. & NELSON, J. D. (1977). A pharmacologic evaluation of penicillin in children with purulent meningitis. *New England Journal of Medicine* **297**, 410–413.

JACOBS, M. R., KOORNHOF, H. J., ROBINS-BROWNE, R. M, STEVENSON, C. M., VERMAAK, Z. A., FREIMAN, I., MILLER, G. B., WITCOMB, M. A., ISAACSON, M., WARD, J. & AUSTRIAN, R. (1978). Emergence of multiply resistant pneumococci. *New England Journal of Medicine* **299**, 735–740.

LIÑARES, J., GARAU, J., DOMINGUEZ, C. & PEREZ, J. L. (1983). Antibiotic resistance and serotypes of *Streptococcus pneumoniae* from patients with community-acquired pneumococcal disease. *Antimicrobial Agents and Chemotherapy* **23**, 545–547.

MANCEBO, J., DOMINGO, P., NET, A. & NOLLA, J. (1985). Existencia de cepas de meningococo resistentes a penicilina. *Medicina Clinica* **84**, 543–544.

SÁEZ-NIETO, J. A. (1985). Informe de la activitad del Laboratorio de Referencia de Meningococos (1978–1984). *Instituto Nacional de Sanidad*. Ministerio de Sanidad y Consumo, pp. 1–43. Madrid. Spain.

SÁEZ-NIETO, J. A., FENOLL, A., VAZQUEZ, J. & CASAL, J. (1982). Prevalence of maltose-negative *Neisseria meningitidis* variants during an epidemic period in Spain. *Journal of Clinical Microbiology* **15**, 78–81.

SÁEZ-NIETO, J. A., GARCIA-BARRENO, B., LOPEZ-GALINDEZ, C. & CASAL, J. (1981*a*). Meningitis meningocócica en España (1978–1980). II. Serotipos y patrones electroforéticos en gel de poliacrilamida. *Revista de Sanidad e Higiene Pública* **55**, 1295–1308.

SÁEZ-NIETO, J. A., LLAGER, A., CATALA, F., FENOLL, A. & CASAL, J. (1981*b*). Meningitis meningocócica en España (1978–1980). I. Estudios epidemiológicos, serogrupos y sensibilidad a antimicrobianos. *Revista de Sanidad e Higiene Pública* **55**, 831–860.

SAEZ-NIETO, J. A., VAZQUEZ, J. & CASAL, J. (1983). Meningitis meningocócica en España (1978–1981). III. Susceptibilidad de cepas de miningococo aislados de enfermos y portadores frente a los principales agentes usados en el tratamiento y la quimioprofilaxis de la infeccion meningocócica. *Revista de Sanidad e Higiene Pública* **57**, 587–602.

SCRIBNER, R. K., WEDRO, B. C., WEBER, A. H. & MARKS, M. I. (1982). Activities of eight beta-lactam antibiotics and seven antibiotic combinations against *Neisseria meningitidis*. *Antimicrobial Agents and Chemotherapy* **21**, 678–680.

WARD, J. (1981). Antibiotic-resistant *Streptococcus pneumoniae*: clinical and epidemiological aspects. *Review of Infectious Diseases* **3**, 254–266.