Epidemics of serogroup A *Neisseria meningitidis* of subgroup III in Africa, 1989–94

M. GUIBOURDENCHE[1], E. A. HØIBY[2], J.-Y. RIOU[1], F. VARAINE[3], C. JOGUET[4] AND D. A. CAUGANT[2]*

[1]*Institut Pasteur, Paris, France*
[2]*WHO Collaborating Centre for Reference and Research on Meningococci, National Institute of Public Health, Geitmyrsveien 75, N-0462 Oslo, Norway*
[3]*Epicentre, Paris, France*
[4]*Médecins Sans Frontières, Brussels, Belgium*

SUMMARY

A total of 125 strains of *Neisseria meningitidis* recovered in the course of outbreaks from patients with systemic disease in 11 African countries between 1989 and 1994 were analysed by serogrouping, serotyping and multilocus enzyme electrophoresis. Of the 125 patient strains 115 (92%) belonged to the clone-complex of serogroup A meningococci, designated subgroup III. Among the remaining strains, 4 were also serogroup A, but belonged to the clonal groups I and IV-1 (2 strains each), whilst 6 strains (4 serogroup C and 2 serogroup W135) represented clones of the ET-37 complex. Our results indicated that the second pandemic caused by clones of subgroup III is still spreading in Africa. Towards the West it has reached Niger, Mali, Guinea and The Gambia, and towards the South, the Central African Republic, Uganda, Rwanda, Burundi, Tanzania and Zambia.

INTRODUCTION

Epidemic waves of meningococcal meningitis occur periodically in Africa every 5–10 years, and usually encompass the Sahel zone of sub-Saharan Africa, called the 'meningitis belt' [1]. The responsible organisms are serogroup A *Neisseria meningitidis*. Morbidity rate in the course of an epidemic may exceed 500 per 100000 [2].

Representative strains from all major serogroup A meningococcal epidemics that occurred worldwide since 1960, as well as a number of serogroup A strains from endemic disease periods have been investigated using multilocus enzyme electrophoresis [3, 4]. A total of 84 electrophoretic types (ETs), which could be classified into 9 clone-complexes or subgroups, have

* Correspondence.

been recognized [4]. Strains belonging to 3 of these subgroups have been identified as the cause of epidemics in Africa.

Clones of subgroup I caused epidemics in North Africa and in countries of the meningitis belt from the beginning of the 1960s and were responsible for epidemics in Nigeria and Rwanda in the late 1970s [3]. Subgroup IV-1 clones have been continuously present in Africa since the early 1960s during inter-epidemic periods, and in the early 1980s were responsible for an epidemic wave in the meningitis belt [5].

Clones of subgroup III, in particular clone III-1, have been responsible for 2 pandemics, both starting in China, 15 years apart [4]. While the first pandemic hit Europe and Brazil [3], the second pandemic that started in the 1980s in China and Nepal, reached Saudia Arabia via pilgrims from East-Asia to Mecca

in August 1987 [6]. Seven thousand cases of meningo-coccal disease occurred during the 1987 Haj pil-grimage, and then many cases were reported among pilgrims returning to their country; in all cases clone III-1 was identified [7–9].

While in industrialized countries, the bacteria did not spread beyond the returning pilgrims and their close-contacts, the introduction of subgroup III into countries of the meningitis belt of Africa, where it had never been recorded prior to the Mecca outbreak, led to major epidemics in 1988–9 in Ethiopia, Chad, Sudan, as well as in Kenya [6, 10–12].

Following the epidemic wave of 1988–9 caused by clone III-1, a number of epidemics occurred in subsequent years in various African countries. We report the characterization of the strains responsible for these epidemics and show that clones of subgroup III have been spreading in Africa, reaching regions to the West and South, well outside the traditional meningitis belt.

MATERIALS AND METHODS

Cerebrospinal fluid samples

Non selective Trans-Isolate media [13] were prepared in Oslo and sent to Médecins Sans Frontières/ Epicentre in Paris and Médecins Sans Frontières in Brussels, together with syringes for inoculation and ventilation, and instructions for use. The media were then distributed to the physicians on mission in Africa. Samples of cerebrospinal fluids (CSF) from patients in Africa were directly inoculated into Trans-Isolate transport media, as described by Ajello and colleagues [13] and sent as rapidly as possible to the WHO Collaborating Centre for Reference and Re-search on Meningococci in Oslo.

Bacterial strains

CSF samples in Trans-Isolate media were plated on chocolate agar and on chocolate agar with colimycin 7·5 mg/l, lincomycin 0·5 mg/l, amphotericin B 1·0 mg/l, and trimethoprim 5·0 mg/l. Plates were incubated at 35 °C in 10 % CO_2 for up to 3 days, and meningococci were identified by standard methods [14]. One colony of *N. meningitidis* from each CSF sample was subcultured twice, each time from a new single colony, and preserved at −70 °C until further analysis. Fifty-one meningococcal strains were obtained in that way.

In addition, in some instances, bacterial identi-fication and serogrouping were performed in the country of origin. The patient strains ($n = 74$) were then sent to the laboratories in Paris or in Oslo, either in Vandekerkove's medium [15] (64 isolates) or in Trans-Isolate medium (10 isolates). Meningococci were preserved either at −70 °C or lyophilized.

Serogrouping and serotyping

Serogroups were determined in Oslo using the ELISA technique and monoclonal antibodies specific for the A, B, C, W135, Y capsular polysaccharides [16] and in Paris by agglutination with immunosera against the 12 serogroups that have been described [14]. Sero-typing and subtyping were performed using mono-clonal antibodies in ELISA or in a dot-blot method [17]. Monoclonal antibodies were kindly provided by Drs J. T. Poolman and W. D. Zollinger. The mono-clonal antibodies against serotype 21 were used only in Oslo.

Susceptibility to antibiotics

Testing of antibiotic susceptibility was performed as previously described [14, 16].

Electrophoresis of enzymes

Multilocus enzyme electrophoresis was performed as described [18, 19], with the exception that the unknown dehydrogenase was not analysed for the strains studied in France. Each isolate was charac-terized by its combination of alleles at 13–14 enzyme loci. Distinctive multilocus genotypes were designated as electrophoretic types, ETs. ETs were cognated with those previously described [4, 20].

RESULTS

Recovery of meningococcal strains from Trans-Isolate medium

A total of 114 Trans-Isolate media, inoculated in Africa by physicians of Médecins Sans Frontières/ Epicentre, were received by the Oslo laboratory. The samples were plated immediately upon arrival, which occurred 2–4 weeks after inoculation. While con-taminants were frequently seen on plates, no men-ingitis pathogens other than *N. meningitidis* were identified. The frequency of recovery of meningococci

Table 1. *Recovery of* Neisseria meningitidis *strains from Trans-Isolate media inoculated with cerebrospinal fluid samples collected in Africa*

Country	No. of Trans-Isolate media received	No. of positive cultures (%)
Niger	23	8 (35)
Sudan	2	1 (50)
Chad	22	8 (36)
Mali	13	12 (92)
Guinea	31	10 (32)
Uganda	4	1 (25)
Burundi	4	2 (50)
Rwanda	1	1 (100)
Zambia	14	8 (57)
Total	114	51 (45)

Table 2. *Characteristics of 125* Neisseria meningitidis *strains isolated from patients in Africa, 1989–94*

Country	Year of isolation	Subgroup/ clone	Serological characteristics	No. of isolates
Morocco	1989	III-1	A:4:P1.9	21
		III, other clone	A:4:P1.9	2
		I	A:NT:P1.10	2
Niger	1991	III-1	A:4,21:P1.9	7
		ET-37 complex	C:2a:-	1
Central Afr. Rep.	1992	III-1	A:4:P1.9	23
		IV-1	A:4:P1.7	1
Sudan	1993	III-1	A:4:P1.9	1
Chad	1993–4	III-1	A:4,21:P1.9	16
Mali	1993–4	III-1	A:21:P1.9	7
		IV-1	A:21:P1.7	1
		ET-37 complex	C:2a:-; W:2a:P1.2	4
Guinea	1993	III-1	A:21:P1.9	3
		III, other clone	A:21:P1.9	6
		ET-37 complex	C:2a:-	1
Cameroon	1993–4	III-1	A:4:P1.9	12
Uganda	1994	III-1	A:21:P1.9	1
Burundi	1992	III-1	A:4,21:P1.9	7
Rwanda	1993	III-1	A:21:P1.9	1
Zambia	1993	III-1	A:(4),21:P1.9	8

in samples from different countries is shown in Table 1. Overall, meningococcal growth was obtained from 51 samples (45%). The percentage of recovery was exceptionally high among the samples from Mali (12 out of 13), and we thus question whether those media were inoculated with CSF or with meningococci isolated in the laboratory in Bamako. Omitting the samples from Mali, the percentage of isolation of meningococci from CSF in Trans-Isolate media was 39%. There was no correlation between the delay before culture and the recovery of meningococci from the samples.

Clone identification and other characteristics of the strains

Table 2 summarizes the origin and the characteristics of the 125 patient strains examined. The analysis of the 24 strains from the Central African Republic has been published elsewhere [21]. All but 6 of the strains (95%) were serogroup A. The 6 exceptional strains were serogroup C (4 strains; 1 from Niger, 2 from Mali, and 1 from Guinea) and serogroup W135 (2 strains from Mali). All these non-serogroup A strains were serotype 2a and represented clones of the ET-37 complex [20], regardless of their serogroup (Table 3). Among the 119 serogroup A strains, only 4 did not belong to subgroup III. Two subgroup I strains (A:NT:P1.10) were identified in Morocco in 1989 and subgroup IV-1 strains were found in the Central African Republic in 1992 and Mali in 1994 (1 strain each). Thus, 115 (92%) of the 125 patient strains collected in Africa between 1989 and 1994 belonged to 1 of 3 clones of subgroup III (Table 3).

All subgroup I and subgroup III strains and all but one of the ET-37 complex strains tested were resistant to sulphonamides, while the strains of clone IV-1 were susceptible to sulphonamides. No decreased sensitivity to penicillin G nor resistance to other antibiotics was found among the strains analysed.

DISCUSSION

Following the introduction of subgroup III strains to Africa in August 1987, major epidemics occurred in Chad, Ethiopia, Sudan and Kenya, in 1988 and 1989, and subgroup III strains of *N. meningitidis* were identified as the responsible organism [6, 10–12]. In 1989, epidemics of meningitis were also reported in Tanzania, Nigeria, Benin, Togo and Cameroon [22–24], but the responsible bacteria were not identified.

From December 1989 to April 1990 an epidemic of meningitis reached Morocco, mainly the southern part of the country [25]. Twenty-three of the 25 isolated strains belonged to subgroup III, while the

Table 3. *Allele profiles of the meningococcal isolates from Africa, 1989–94*

Clone	Allele at indicated enzyme locus*													
	ME	G6P	PEP	IDH	ACO	GD1	GD2	ADH	FUM	ALK	IP1	IP2	ADK	UDH
Subgroup III														
III-1	1	4	5	6	4	1	3	2	1	8	2	3	2	2
Other clone, Morocco	0·5	4	5	6	4	1	3	2	1	8	2	3	2	ND
Other clone, Guinea	1	4	5	6	4	1	3	3	1	8	2	3	2	2
Clone IV-1	1	3	2	6	4	1	1	2	1	8	2	3	2	2
Subgroup I	1	3	2	3	4	1	3	3	1	8	2	5	2	ND
ET-37 complex														
Niger, Guinea, Mali (C:2a:-)	4	3	5	5	2	1	4	0	1	8	2	3	2	3
Mali (W:2a:P1.2)	4	3	4	5	2	1	4	1	1	8	2	3	2	5

* ME, malic enzyme; G6P, glucose 6-phosphate dehydrogenase; PEP, peptidase; IDH, isocitrate dehydrogenase; ACO, aconitase; GD1 and GD2, two glutamate dehydrogenases; ADH, alcohol dehydrogenase; FUM, fumarase; ALK, alkaline phosphatase; IP1 and IP2, two indophenol oxidases; ADK, adenylate kinase; UDH unknown dehydrogenase.

remaining 2 belonged to subgroup I, which had already been identified in that country in the 1960s [4]. A study of meningococcal carriage was performed in July 1989 in a military camp situated in the region of Casablanca, and a carriage rate of 4·3% was observed [25]. All the 65 strains isolated were identical and represented a clone of subgroup III identified in 2 of the 25 patients from Morocco in 1989 included in this study (Guibourdenche and Riou, unpublished data). After the outbreak, cases of meningococcal meningitis caused by subgroup III strains continued to appear sporadically in Morocco until 1994 (Benbachir and Caugant, unpublished data).

In January 1990, an outbreak of meningococcal disease occurred in Uganda [26], but no strains were analysed. The next epidemic episode occurred in Niger between November 1990 and April 1991. The number of recorded cases was 6278 for the whole country (incidence 79/100000) with a case fatality rate of 10·6%. The regions of Zinder and Maradi were the worst affected, with a total of 4242 cases (incidence 138/100000). The attack rate was highest in the age-group 5–14 years old. A large proportion of the population in these regions had been vaccinated in the course of the previous epidemic, in 1986. The 8 strains we recovered were from CSF of patients in Zinder. Seven of these were clone III-1 and one was serogroup C of the ET-37 complex, suggesting that the epidemic was not due to a single clone, although it is likely that a large part of the epidemic was due to subgroup III organisms.

From March to November 1991, an outbreak of serogroup A meningococcal disease occurred in South Africa among refugees from Mozambique. All 10

isolates we received belonged to clone I-1 [unpublished data], a clone previously identified in many African countries, inclusive of South Africa [4]. Thus, this outbreak was not related to the epidemic wave caused by strains of subgroup III.

No outbreak was reported in Africa by the World Health Organization Weekly Epidemiological Record between the epidemic in Uganda in early 1990 [26] and the resurgence of clone III-1 in the Central African Republic in the early 1992 [21], suggesting that with the exception of the epidemic in Niger, this 2-year period was marked by low incidence of disease.

In 1992, in addition to the outbreak in the Central African Republic which has been described elsewhere [27], an epidemic of meningococcal meningitis occurred in Burundi from July to October, with a total of 2272 cases (incidence 41/100000) and a recorded case fatality rate of 4·2%. This was the first epidemic reported in Burundi since 1981, and the highest attack rate was in 30–34 year olds. The seven strains analysed were all clone III-1, demonstrating the extension towards the South of the meningitis belt. In the first 9 months of 1992, 4279 cases of cerebrospinal meningitis were also reported in Tanzania, but no strain from that country was available [28].

From December 1992 to March 1993 cases of meningococcal meningitis occurred in Sudan, but an epidemic was not confirmed. The only isolate that was recovered was clone III-1. Between January and March 1993, parts of Chad and parts of Guinea were involved in a severe epidemic with an estimated incidence of 260/100000 and 136/100000, respectively. All strains from Chad, and 9 of the 10 strains from Guinea that we analysed belonged to subgroup

III. However, 6 of the 9 strains from Guinea represented a different clone of subgroup III.

In Zambia, between 26 July and 19 September 1993 an epidemic was declared in the district of Ndola, in the Copper Belt Province, with 1381 reported cases. The attack rate in that period was 330/100000 and the case fatality rate was 10%. The highest attack rate was in individuals over 14 years old. All 8 strains isolated in the course of this epidemic were subgroup III.

In the course of 1993–4, cases of meningococcal disease caused by subgroup III organisms also occurred in Cameroon, Uganda and Rwanda, but we have no information concerning the severity of the situation in those countries. Mali was also reached by strains of subgroup III in 1993. While the few strains of serogroup A meningococci from Mali isolated between 1989 and 1992 were all subgroup IV-1 (M. Achtman, personal communication), a clonal replacement occurred in 1993–4, with 7 of the 8 serogroup A strains analysed being clone III-1.

Our study demonstrates that the spread of strains of subgroup III in Africa led to epidemics and outbreaks well outside the traditional meningitis belt. The meningitis belt, as described by Lapeysonnie in 1963, has been subsequently enlarged to include Ethiopia, Kenya, Uganda and Rwanda, in the East, and parts of Mali, Gambia, and Senegal in the West in the revised map published by Moore in 1992 [29]. Our data showed that the Central African Republic and Cameroon, in the South of the Sahelian region, also experienced outbreaks after the introduction of clones of subgroup III, as did Burundi, Zambia and probably Tanzania, even further south. This extension could be linked to the climatic changes towards desert conditions experienced by that region. A strong correlation of humidity and a low potential for epidemic meningococcal disease has been recently documented [30]. However, severe serogroup A epidemics have been reported in Africa before, well outside the boundaries of the meningitis belt [31], and it is possible that the apparent extension of the meningitis belt is a result, at least in part, of improved information.

Ajello and colleagues [13] reported a recovery of the aetiological agents in 73% of the Trans-Isolate media inoculated with CSF in Africa. Under similar conditions, we obtained growth of *N. meningitidis* in about 40% of the media. This method of sending CSF proved very useful to study the organisms causing meningitis epidemics in regions of Africa that are difficult to get access to, where the local doctor has no means to dispatch the CSF to a laboratory or to isolate the strains.

ACKNOWLEDGEMENTS

REFERENCES

1. Lapeysonnie L. La méningite cérébrospinale en Afrique. Bull WHO 1963; 28 (Suppl): 53–114.
2. Achtman M. Molecular epidemiology of epidemic bacterial meningitis. Rev Med Microbiol 1990; 1: 29–38.
3. Olyhoek T, Crowe BA, Achtman M. Clonal population structure of *Neisseria meningitidis* serogroup A isolated from epidemics and pandemics between 1915 and 1983. Rev Infect Dis 1987; 9: 665–95.
4. Wang J, Caugant DA, Li X, et al. Clonal and antigenic analysis of serogroup A *Neisseria meningitidis* with particular reference to epidemiological features of epidemic meningitis in China. Infect Immun 1992; 60: 5267–82.
5. Achtman M, Kusecek B, Morelli G, et al. A comparison of the variable antigens expressed by clone IV-1 and subgroup III of *Neisseria meningitidis* serogroup A. J Infect Dis 1992; 165: 53–68.
6. Moore PS, Reeves MW, Schwartz B, Gellin BG, Broome CV. Intercontinental spread of an epidemic group A *Neisseria meningitidis* strain. Lancet 1989; ii: 260–2.
7. Moore PS, Lee LH, Telzak EE, Ajello GW, Broome CV. Group A meningococcal carriage in travellers returning from Saudi Arabia. JAMA 1988; 260: 2686–9.
8. Jones DM, Sutcliffe EM. Group A meningococcal disease in England associated with the Haj. J Infect 1990; 21: 21–5.
9. Riou JY, Caugant DA, Selander RK, Poolman JT, Guibourdenche M, Collatz E. Characterization of *Neisseria meningitidis* serogroup A strains from an outbreak in France by serotype, serosubtype, multilocus enzyme genotype and outer membrane protein pattern. Eur J Clin Microbiol Infect Dis 1991; 10: 405–9.
10. Tekle Haimanot R, Caugant DA, Fekadu D, et al. Characteristics of serogroup A *Neisseria meningitidis* responsible for an epidemic in Ethiopia. Scand J Infect Dis 1990; 22: 171–4.
11. Salih MAM, Danielsson D, Bäckman A, Caugant DA, Achtman M, Olcén P. Characterization of epidemic and

non-epidemic *Neisseria meningitidis* serogroup A strains from Sudan and Sweden. J Clin Microbiol 1990; **28**: 1711–9.

12. Pinner RW, Onyango F, Perkins BA, et al. Epidemic meningococcal disease in Nairobi, Kenya, 1989. J Infect Dis 1992; **166**: 359–64.

13. Ajello GW, Feeley JC, Hayes PS, et al. Trans-Isolate medium: a new medium for primary culturing and transport of *Neisseria meningitidis, Streptococcus pneumoniae,* and *Haemophilus influenzae.* J Clin Microbiol 1984; **20**: 55–8.

14. Riou JY, Guibourdenche M. Laboratory methods: *Neisseria* and *Branhamella.* Paris: Editions Pasteur, 1993.

15. Vandekerkove M, Bradstreet P, Faucon R, Beucali S. Milieu de transport pour méningocoques. Ann Inst Pasteur 1967; **113**: 260–3.

16. Caugant DA, Høiby EA, Magnus P, et al. Asymptomatic carriage of *Neisseria meningitidis* in a randomly sampled population. J Clin Microbiol 1994; **32**: 323–30.

17. Wedege E, Høiby EA, Rosenqvist E, Frøholm LO. Serotyping and subtyping of *Neisseria meningitidis* isolates by co-agglutination, dot-blotting and ELISA. J Med Microbiol 1990; **31**: 195–201.

18. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 1986; **51**: 873–84.

19. Caugant DA, Bol P, Høiby EA, Zanen HC, Frøholm LO. Clones of serogroup B *Neisseria meningitidis* causing systemic disease in the Netherlands, 1958 through 1986. J Infect Dis 1990; **162**: 867–74.

20. Wang J, Caugant DA, Morelli G, Koumaré B, Achtman M. Antigenic and epidemiological properties of the ET-37 complex of *Neisseria meningitidis.* J Infect Dis 1993; **167**: 1320–9.

21. Guibourdenche M, Caugant DA, Hervé V, et al. Characteristics of serogroup A *Neisseria meningitidis* strains isolated in the Central African Republic in February 1992. Eur J Clin Microbiol Infect Dis 1994; **13**: 37–41.

22. World Health Organization. Meningitis. Wkly Epidem Rec 1989; **22**: 171–2.

23. World Health Organization. Meningitis. Wkly Epidem Rec 1989; **29**: 225.

24. World Health Organization. Meningitis. Wkly Epidem Rec 1989; **37**: 286.

25. Nejmi S, Belhaj M, Guibourdenche M, Riou JY. Etude de quatre vingt dix souches de *Neisseria meningitidis* de sérogroupe A isolées du liquide céphalorachidien (25) et du rhinopharynx (65) au Maroc (décembre 1989–avril 1990). Path Biol 1992; **40**: 993–8.

26. World Health Organization. Meningitis. Wkly Epidem Rec 1990; **4**: 26.

27. World Health Organization. Cerebrospinal meningitis in the Central African Republic. Wkly Epidem Rec 1992; **67**: 333–40.

28. World Health Organization. Meningitis. Wkly Epidem Rec 1992; **43**: 323.

29. Moore PS. Meningococcal meningitis in sub-Saharan Africa: a model for the epidemic process. Clin Infect Dis 1992; **14**: 515–25.

30. Cheesbrough JS, Morse AP, Green SDR. Meningococcal meningitis and carriage in western Zaire: a hypoendemic zone related to climate? Epidemiol Infect 1995; **114**: 75–92.

31. Peltola H. Meningococcal disease: still with us. Rev Infect Dis 1983; **5**: 71–91.