the patient was initially treated for toxoplasmosis based on serologic data. During the second toxoplasmic episode, it is unclear whether toxoplasmic reactivation was initiated by the surgical procedure or had already started before surgery. The patient was receiving broad-spectrum antibacterial therapy at the time of sampling of the peritoneal fluid. Therefore, negative culture for bacteria cannot rule out the possibility of a bacterial peritonitis, although the cell count of the peritoneal fluid makes this hypothesis unlikely. Whilst detection of DNA by PCR is not absolute evidence for the presence of viable *T. gondii*, rapid clinical improvement with resolution of the fever after institution of specific antiprotozoal therapy supports the diagnosis of active toxoplasmosis. The fact that the patient was treated with pyrimethamine at the time of reactivation confirms previous reports that pyrimethamine alone is not always efficient in preventing toxoplasmosis in immunocompromised patients [6], and further emphasizes the importance of DNA amplification, since diagnosis of toxoplasmosis is unlikely to be established by mice inoculation or cell culture in a patient receiving antiprotozoal therapy.

J. Lopez[1], *D. Caillot*[2], *O. Vagner*[1], *P. Chalabreysse*[3], *B. Cuisenier*[1], *O. Casasnovas*[2], *A. Bonnin*[1]*

[1]Laboratoire de Parasitologie et Mycologie, [2]Service d'Hématologie Clinique, and [3]Laboratoire d'Anatomie Pathologique, Centre Hospitalier Universitaire, Dijon, France

*Tel: +33 3 80 29 36 03 Fax: +33 3 80 29 32 80

Accepted 3 December 1997

References

1. Albrecht H, Skorde J, Arasteh K, et al. Disseminated toxoplasmosis in AIDS patients. Report of 16 cases. Scand J Infect Dis 1995; 27: 71–4.
2. Khalifa el Sayed K, Roth A, Roth B, Arasteh KN, Janitschke K. Value of PCR for evaluating occurrence of parasitemia in immunocompromised patients with cerebral and extracerebral toxoplasmosis. J Clin Microbiol 1994; 32: 2813–19.
3. Bretagne S, Costa JM, Vidaud M, Tran Van Nhieu J, Fleury-Feith J. Detection of *Toxoplasma gondii* by competitive DNA amplification of bronchoalveolar lavage samples. J Infect Dis 1993; 168: 1585–8.
4. Hohlfeld P, Daffos F, Costa JM, Thulliez P, Forestier F, Vidaud M. Prenatal diagnosis of congenital toxoplasmosis with a polymerase-chain-reaction test on amniotic fluid. N Engl J Med 1994; 331: 695–9.
5. Pinon JM, Foudrinier F, Mougeot G, et al. Evaluation of risk and diagnostic value of quantitative assays for anti *Toxoplasma gondii* immunoglobulin A (Ig A), IgE, and IgM and analytical study of specific IgG in immunodeficient patients. J Clin Microbiol 1995; 33: 878–84.
6. Leport C, Chêne C, Morlat P, et al. Pyrimethamine for primary prophylaxis of toxoplasmic encephalitis in patients with HIV infection: a double-blind, randomized trial. J Infect Dis 1996; 173: 91–7.

Surveillance of meningococcal infections in Belgium

Clin Microbiol Infect 1998; 4: 224–228

Meningococcal disease remains an important public-health problem in both developing [1] and industrialized countries [2]. Before 1990, the annual incidence of meningococcal disease in Belgium fluctuated around one case per 100 000 inhabitants. However, since the early 1990s an increase in the incidence of meningococcal disease has been seen.

Meningococci are subdivided into serotypes based on the immunologic properties of several outer-membrane proteins and lipopolysaccharides [3], and development of monoclonal antibodies against those antigens has significantly contributed to a better understanding of the epidemiology of meningococcal disease [3].

In Belgium, serogroup B is the predominant serogroup causing meningococcal disease, followed by serogroup C. No effective vaccine is currently available for protection against group B meningococcal disease, because the B capsular polysaccharide is not immunogenic in humans [4]. The recent emergence of penicillin-resistant meningococcal strains [5,6] may create additional therapeutic problems.

In the present investigation we describe the spread in Belgium of a serogroup B strain which was first reported in The Netherlands in the early 1980s [7]. Isolates were characterized by traditional serotyping, and their susceptibility patterns to a panel of antimicrobial agents were determined. Extensive phenotyping (multilocus enzyme electrophoresis) and genotyping (PCR and pulsed-field gel electrophoresis analyses) were performed on these and other strains [8].

Meningococcal infections are notifiable in Belgium. Trends of meningococcal disease have been derived from epidemiologic and laboratory data sets that are complementary but are maintained separately. On the one hand, the Federal Belgian Health Inspection receives physician notifications; data include age, gender, date and location. On the other hand, the Belgian Meningococcal Reference Center at the Scientific Institute for Public Health–Louis Pasteur (Brussels) receives isolates of *Neisseria meningitidis* from

the Belgian diagnostic laboratories for confirmation and determination of serotype, subtype, and susceptibility pattern. Since 1985, the annual number of meningococcal isolates submitted to the Belgian Meningococcal Reference Center has exceeded the number of cases reported [9]. It is generally accepted that the number of submissions to the Belgian Meningococcal Reference Center is highly representative for the number of cases of meningococcal disease in Belgium.

From 1990 to 1995, isolates from 769 patients (429 males, 324 females, 16 of unspecified gender; male/female ratio 1.32) were submitted to the Belgian Meningococcal Reference Center. Seven hundred and fifty-three strains were recovered from cerebrospinal fluid or blood, and 16 strains were throat or skin isolates of patients with meningococcal disease.

Serogroups were determined by slide agglutination with commercially available rabbit antisera specific for the A, C, X, Y, Z, W135 (Murex Diagnostics, Dartford, UK) and 29E (Diagnostics Pasteur, Marnes-la-Coquette, France) capsular polysaccharides [10]. For serogroup B, a monoclonal antibody (Murex Diagnostics, Dartford, UK) was used.

Serotyping and subtyping with monoclonal antibodies (J.T. Poolman, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands) was carried out using an enzyme-linked immunoblot assay [11] or whole cell ELISA [12].

In Belgium, an epidemic elevation of the number of cases of meningococcal disease was observed between 1969 and 1975. In 1972, the epidemic reached its peak incidence of 5.3 cases per 100 000 inhabitants [13]. Afterwards, the incidence of disease decreased and fell to normal inter-epidemic proportions of one case per 100 000 inhabitants per year. Except for a small augmentation in 1980, the incidence has fluctuated around this value. However, during the past 6 years, the incidence of meningococcal disease in Belgium, calculated from the submission of meningococcal isolates to the Belgian Meningococcal Reference Center, has gradually increased from 0.8 cases per 100 000 inhabitants in 1990 to 2.0 cases per 100 000 inhabitants in 1995. This increase is particularly due to a rise in the proportion of serogroup B strains. Serogroup B meningococci always accounted for more than 60% of the cases of meningococcal disease, but in recent years their proportion has increased to up to 80% of the cases. The distribution of serotypes and subtypes of all 568 serogroup B isolates obtained during 1990–95 is shown in Table 1. Amongst the serogroup B strains, the increase of serotype 4 isolates was the most obvious, their relative frequency increasing from 4% in 1990 to 74% in 1995. Serotype 4 strains were mostly associated with subtype P1.4 group B isolates. The proportion of subtype P1.4 increased from 6% in 1990 to 48% in 1995 (Table 1). Strains with phenotype B:4:P1.4 currently cause 32.5% of the cases of meningococcal disease; however, B:non-typeable:P1.4 strains account for an additional 3.5% (total of 36.0%).

Strains with phenotype B:4:P1.4 were first isolated in The Netherlands in 1980 [7]. Until 1984, they were predominantly recovered in the southwestern part of The Netherlands in the vicinity of Rotterdam and Breda. Thereafter, the phenotype dispersed throughout the country, but the incidence remained most pronounced in the southern provinces [14]. In Belgium, an increase of meningococcal infections, with B:4:P1.4 and B:non-typeable:P1.4 as predominant phenotypes, was first noticed in the province of Antwerp, near the border with The Netherlands. Later, a similar trend was seen in other provinces of Belgium, particularly East and West Flanders. In 1994, the Walloon provinces were reached, and in 1995 phenotypes B:4:P1.4 and B:non-typeable:P1.4 were encountered in all Belgian provinces (Figure 1). Consequently, dispersion of the Dutch B:4:P1.4 strain in Belgium can be hypothesized.

Table 1 Distribution of serotypes and subtypes among 568 serogroup B meningococci isolated from patients with meningococcal disease in Belgium between 1990 and 1995

	Year of isolation					
	1990	1991	1992	1993	1994	1995
No of isolates tested	53	77	91	94	95	158
Serotypes (%)						
1	–	3	2	6	2	4
2a	4	3	2	1	1	1
2b	6	3	4	4	5	–
4	4	1	7	38	53	74
14	2	–	2	2	–	1
15	9	10	7	9	4	5
16	–	4	3	–	–	–
Non-serotypeable	75	77	74	40	34	14
Subtypes (%)						
P1.1	–	6	1	–	–	–
P1.1,7	–	–	3	2	8	4
P1.2	13	10	3	4	3	1
P1.2,5	–	–	–	–	–	4
P1.4	6	21	32	40	45	48
P1.5	–	–	–	2	–	3
P1.6	9	–	–	1	–	2
P1.7	–	1	–	3	2	3
P1.7,16	–	–	3	6	4	4
P1.9	2	4	2	–	3	1
P1.10	2	1	10	11	11	–
P1.12	–	–	2	4	–	2
P1.13	–	–	–	9	8	9
P1.14	–	–	1	3	1	5
P1.15	9	26	7	3	4	6
P1.16	8	10	5	3	1	4
Non-subtypeable	51	19	30	7	8	4

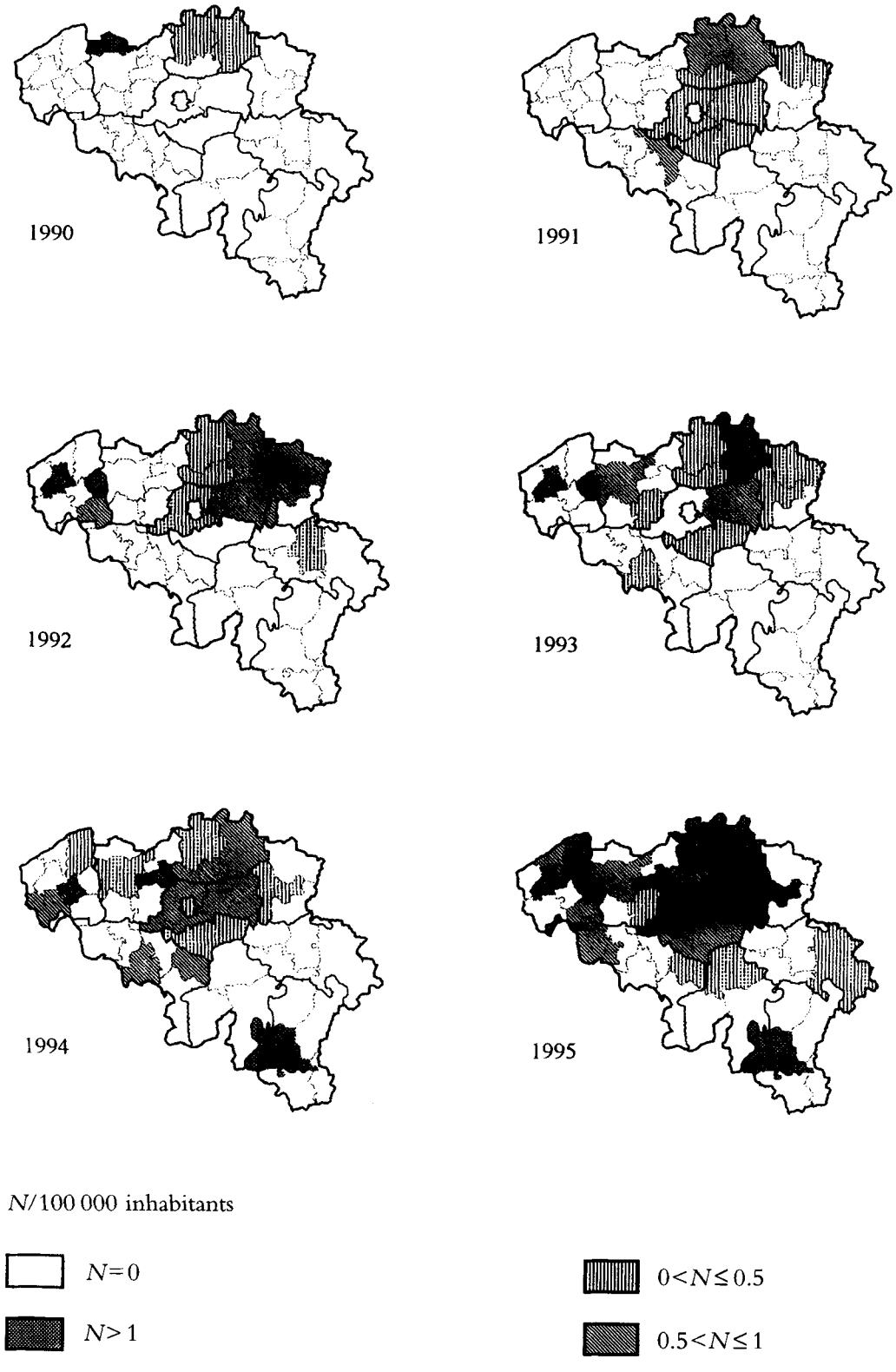

Figure 1 Evolution of the geographic distribution of isolates belonging to the phenotypes B:4:P1.4 or B:non-typeable: P1.4 in the period 1990–95. The incidence rate per 100 000 of inhabitants is given.

These and other [8] data suggest that the elevated number of meningococcal infections is due to the introduction of a new strain of *N. meningitidis* (B:4:P1.4/B:non-typeable:P1.4) in Belgium since the beginning of the 1990s. The introduction of a new strain in a susceptible population was previously shown to cause a rise in the number of meningococcal infections [7]. In The Netherlands, the incidence of serotype B:4 rose from 11% in 1980 to 43% in 1990, and the incidence of the subtype P1.4 increased from 3% in 1980 to 41% in 1990 [7]. However, the highest prevalence of strain B:4:P1.4 was only 21%. As a consequence, B:4:P1.4 alone could not be held responsible for the increase of meningococcal disease in The Netherlands after 1982 [7]. Also in England and Wales, B:4:P1.4 strains have become established in the past 5 years and now account for a quarter of all group B infections (25% in 1995) [15].

Age distribution

Meningococcal disease predominates in the age group of 0–4 years, accounting for 38–49% of the annual registered number of cases. From 1990 to 1995, children less than 1 year of age accounted for 13.0%, 17.7%, 15.1%, 18.1%, 17.3%, and 12.5%, respectively, of the cases of meningococcal disease. However, from 1993 onwards, a secondary peak was observed in the age group from 15 to 19 years. The incidence among children aged between 15 and 19 years old increased from 0.9 per 100 000 inhabitants in 1991 (95% confidence interval (95% CI): 0.2–1.7) to 6.2 per 100 000 inhabitants in 1995 (95% CI: 4.2–8.2). This difference of 5.3 was statistically significant (95% CI: 3.2–7.4). A shift in age distribution from younger to older age categories has been observed before and was explained by a coinciding shift of the serogroup and/or serosubtype distribution [7]. Indeed, in Belgium, serogroup B has become more prevalent in recent years, and the introduction of phenotypes not previously encountered has also been seen (B:4:P1.4 and B:non-typeable:P1.4).

Antibiotic susceptibility testing

A random sample of 420 clinical isolates stratified by serogroup and year of isolation was selected for susceptibility testing by E-test (AB Biodisk, Sölna, Sweden). The use of the E-test for determining antibiotic susceptibility of *N. meningitidis* was validated by Hughes et al [16]. They found that E-test MICs were within ± 1 $\log_2$ dilution of the MICs by agar dilution.

The susceptibilities to five antimicrobial agents, i.e. penicillin, rifampicin, sulfadiazine, ciprofloxacin and ceftriaxone, were determined. The E-test was performed as previously described by Blondeau and Yaschuk [17]. Definitions of susceptibility were as follows: for penicillin, MIC ≤ 0.06 mg/L indicated susceptibility, MIC >0.06–1 mg/L relative resistance, and MIC >1 mg/L resistance [6]; for all other antimicrobial agents, published NCCLS breakpoint definitions were used [18].

Although penicillin remains the drug of choice for serious meningococcal disease, the drug appears to be less active because of emerging resistance [17,19–21]. The first meningococcal strain isolated in Belgium with a reduced susceptibility to penicillin (0.5 mg/L) was reported in 1993 [22]. We observed a reduced susceptibility to penicillin (MIC >0.06–0.125 mg/L) [16,17] for 27 strains (6.4%). The strains showed the following MICs: 0.064 mg/L for 18 isolates, 0.094 mg/L for eight isolates and 0.125 mg/L for one isolate. High-level resistance to sulfadiazine (MIC >256 mg/L) was observed in 80 strains (19.0%). No association was found between sulfadiazine resistance and serotype or subtype. All 420 isolates were susceptible to rifampicin, ciprofloxacin and ceftriaxone.

In conclusion, we hypothesize that the increase in the incidence of meningococcal disease in Belgium is due to the introduction of a new strain of *N. meningitidis* (B:4:P1.4) in the susceptible Belgian population since the early 1990s. Phenotype B:4:P1.4 strains were first encountered in The Netherlands, and have caused disease their since the early 1980s. We suggest that these strains dispersed into Belgium from 1990 onwards.

Acknowledgments

We thank Machteld Hauchecorne and Monik Wijdooghe for their excellent technical assistance, Geneviève Ducoffre for her kind help with the figure, and Pierre Van Damme and Jean-Claude Van Der Auwera for statistical advice. P.V. is indebted to the Fund for Scientific Research–Flanders for a position as a postdoctoral research fellow.

The results of this study were presented in part as a poster at the 8th European Congress of Clinical Microbiology and Infectious Diseases held in Lausanne, Switzerland, 25–28 May 1997 (abstract 414) and at the 4th meeting of the European Monitoring group on Meningococci held in Paris, France, 4–7 June 1997 (abstract 18).

Marleen Van Looveren[1],
Françoise Carion[2],
Peter Vandamme[1,3]
Herman Goossens[1]
[1]Department of Microbiology, University Hospital Antwerp, UIA, Antwerp;
[2]Department of Bacteriology, Scientific Institute for Public Health–Louis Pasteur, Brussels;

[3]Department of Microbiology,
University of Ghent,
Ghent, Belgium

Accepted 11 December 1997

References

1. Guibourdenche M, Høiby EA, Riou JY, Variane F, Joguet C, Caugant DA. Epidemics of serogroup A *Neisseria meningitidis* of subgroup III in Africa, 1989–94. Epidemiol Infect 1996; 116: 115–20.
2. Ringuette L, Lorange M, Ryan A, Ashton F. Meningococcal infections in the province of Québec, Canada, during the period 1991 to 1992. J Clin Microbiol 1995; 33: 53–7.
3. Frasch CE, Zollinger WD, Poolman JT. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. Rev Infect Dis 1985; 7: 504–10.
4. Romero JD, Outschoorn IM. Current status of meningococcal group B vaccine candidates: capsular or noncapsular? Clin Microbiol Rev 1994; 7: 559–75.
5. Blondeau JM, Ashton FE, Isaacson M, Yaschuk Y, Anderson C, Ducasse G. *Neisseria meningitidis* with decreased susceptibility to penicillin in Saskatchewan, Canada. J Clin Microbiol 1995; 33: 1784–6.
6. Campos J, Trujillo G, Seuba T, Rodriguez A. Discriminative criteria for *Neisseria meningitidis* isolates that are moderately susceptible to penicillin and ampicillin. Antimicrob Agents Chemother 1992; 5: 1028–31.
7. Scholten RJPM, Bijlmer HA, Poolman JT, et al. Meningococcal disease in the Netherlands, 1958–1990: a steady increase of the incidence since 1982 partially caused by new serotypes and subtypes of *Neisseria meningitidis*. Clin Infect Dis 1993; 16: 237–46.
8. Van Looveren M, Vandamme P, Hauchecorne M, et al. Molecular epidemiology of recent Belgian isolates of *Neisseria meningitidis* serogroup B. Submitted.
9. Carion F. Meningococci: Surveillance in Belgium by the reference laboratory. In: Program and abstracts of the 11th Seminar on Diagnostics and Surveillance of Infectious Diseases. Brussels, Belgium: Institute of Hygiene and Epidemiology, 1995: 21–9.
10. Riou JY, Saliou P, Guibourdenche M, Chalvignac MA. Etude bactériologique et sérologique de 358 souches de *Neisseria* et *Branhamella* isolées en Haute-Volta. Med Mal Infect 1980; 10: 430–6.
11. Calain P, Poolman J, Zollinger W, et al. Serological study of meningococcal isolates in Switzerland and France 1980–1986. Eur J Clin Microbiol Infect Dis 1988; 7: 788–91.
12. Abdillahi H, Poolman JT. Whole-cell ELISA for typing *Neisseria meningitidis* with monoclonal antibodies. FEMS Microbiol Lett 1987; 48: 367–71.
13. De Maeyer S, Seba JM, Reginster G. Epidemiology of meningococcal meningitis in Belgium. J Infect 1981; 3(suppl 1): 63–70.
14. Caugant DA, Bol P, Høiby EA, Zanen HC, Frøholm LO. Clones of serogroup B *Neisseria meningitidis* causing systemic disease in the Netherlands, 1958–1986. J Infect Dis 1990; 162: 867–74.
15. Kaczmarski EB. Meningococcal disease in England and Wales: 1995. CDR Rev 1997; 7: R55–9.
16. Hughes JH, Bredenbach DJ, Erwin ME, Jones RN. E-test as susceptibility test and epidemiologic tool for evaluation of *Neisseria meningitidis* isolates. J Clin Microbiol 1993; 31: 3255–9.
17. Blondeau JM, Yaschuk Y. In vitro activities of ciprofloxacin, cefotaxime, ceftriaxone, chloramphenicol, and rifampin against fully susceptible and moderately penicillin-resistant *Neisseria meningitidis*. Antimicrob Agents Chemother 1995; 39: 2577–9.
18. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M100-S6. Villanova, PA: NCCLS, 1997.
19. Sutcliffe EM, Jones DM, El-Sheikh S, Percival A. Penicillin-insensitive meningococci in the UK. Lancet 1988; i: 657–8.
20. Sáez-Nieto JA, Lujan R, Berrón S, et al. Epidemiology and molecular basis of penicillin-resistant *Neisseria meningitidis* in Spain: a 5-year history (1985–1989). Clin Infect Dis 1992; 14: 394–402.
21. Jackson LA, Tenover FC, Baker C, et al. Prevalence of *Neisseria meningitidis* relatively resistant to penicillin in the United States, 1991. J Infect Dis 1994; 169: 438–41.
22. Brunen A, Peetermans W, Verhaegen J, Robberecht W. Meningitis due to *Neisseria meningitidis* with intermediate susceptibility to penicillin. Eur J Clin Microbiol Infect Dis 1993; 12: 969–70.

First case of *Salmonella hirschfeldii* (paratyphi C) infection of a prosthetic hip

Clin Microbiol Infect 1998; 4: 228–230

Infection after a total hip replacement is a serious complication, often requiring removal of the prosthesis for healing [1]. The commonest etiologic agents are *Staphylococcus aureus* and *Staphylococcus epidermidis*, which account for more than 50% of the pathogens isolated, according to recent series. Other organisms may be involved, especially in hematogenous infections, including Gram-negative bacteria, *Streptococcus*, anaerobic bacteria, diphtheroids, or mycobacteria [1,2]. We report the first case of typhoid salmonella infection of a total hip prosthesis.

A 51-year-old black African man was admitted because of fever, pain of the right hip and a fistula from the joint to the thigh surface. His past medical history was remarkable for several episodes of malaria, and type 2 diabetes mellitus. Eleven years before this admission, he suffered post-traumatic necrosis of the right femoral head, and underwent total hip arthroplasty with a satisfactory postsurgical functional result. Thirty months before admission, the patient suddenly developed a fever, and swelling of the upper lateral part of the right thigh. A hip puncture, performed in Brazzaville,