Use of Single-Dose Ofloxacin To Eradicate Tonsillopharyngeal Carriage of *Neisseria meningitidis*

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After an outbreak of three cases of serogroup B meningococcal disease at a Norwegian college, 84 of 392 (21%) subjects were tonsillopharyngeal carriers of *Neisseria meningitidis*. To eradicate meningococcal carriage, 80 volunteers received a single dose of 400 mg of ofloxacin. Three days after treatment, all 75 evaluable volunteers were culture negative for *N. meningitidis*, and after 7 days none carried the strain that they carried initially, as judged by DNA fingerprinting. A single dose of ofloxacin was found to be 97.2% effective in eradicating carriage of *N. meningitidis* for a period of 33 days. The carriage acquisition rate among treated students was four times higher than that among nontreated noncarriers ($P = 0.02$). After ofloxacin treatment, no case of meningococcal disease occurred for 6 months. Ofloxacin may thus prevent the outbreak and spread of meningococcal disease.

The eradication of *Neisseria meningitidis* from the pharynx and tonsils of carriers is presumed to be important in the management of meningococcal disease outbreaks. Sulfonamides were recommended for this purpose until resistance emerged. Rifampin is considered effective, but it frequently causes side effects (1) and has induced resistance after widespread use (2). Ciprofloxacin has proven highly effective against meningococcal carriage (13, 14), even as a single-dose regimen (5, 6), and resistant strains have not been isolated. Ofloxacin is highly effective against *N. meningitidis* in vitro and has favorable pharmacokinetic properties compared with those of ciprofloxacin. After oral administration, the drug is rapidly absorbed, with an absolute bioavailability of >95%. The penetration to saliva is greater for ofloxacin than for ciprofloxacin (11), and high levels are obtained in the tonsils (16). Furthermore, ofloxacin reaches high intracellular concentrations in phagocytic cells in the respiratory tract, exceeding the extracellular concentration by up to six times (12). Accordingly, a single dose of ofloxacin might be efficient to eradicate meningococcal carriage. The purpose of the present study was to evaluate the efficacy of ofloxacin in eradicating meningococcal carriage from a defined population with the aim of preventing additional cases of meningococcal disease.

During the period from 1988 to 1991, the incidence of meningococcal disease was fairly constant in Norway (10). During the first 3 months of 1992, a fivefold increase in the number of patients ($n = 15$) admitted with meningococcal disease was observed at Haukeland University Hospital in Bergen in comparison with the number in the first 3 months of 1991. Three of these patients were students at Øystese College, and their meningococcal disease occurred within a period of 11 weeks. Øystese is a rural community with 2,270 inhabitants where no other case of meningococcal disease had been observed since 1988. All three patients had typical systemic meningococcal disease including skin bleedings. Two of them were treated intramuscularly with penicillin

before admission. *N. meningitidis* serogroup B was cultured from the pharynxes of two of the patients, and gram-negative diplococci were seen in the cerebrospinal fluid of the third patient. Analysis of acute-phase and convalescent-phase sera from all three patients revealed the typical seroconversion seen in patients with *N. meningitidis* serogroup B disease.

Standardized tonsillopharyngeal swabbing was performed on 343 students and 49 staff personnel by three medical doctors at the Øystese College by using a dry coal-cotton pin. One student resisted swabbing, and four students were absent during the first sampling. The specimens were immediately inoculated onto chocolate agar containing vancomycin (3 g/liter), colimycin (12.35 mg/liter), trimethoprim lactate (5 g/liter), and amphotericin B (10,000 U/liter). After 2 to 4 h, the plates were incubated at 37°C in air containing 5% CO_2 and 80% humidity. The cultures were read after 24 and 48 h. All isolates were identified by oxidase reaction, carbohydrate fermentation (glucose, maltose, sucrose, and lactose), and microscopic appearance. Isolates were tested for γ-glutamylaminopeptidase and polysaccharide production from 5% sucrose. Whole-cell enzyme-linked immunosorbent assay serogrouping with monoclonal antibodies against serogroups A, B, C, and Y was carried out (15). Serotyping was performed correspondingly (18) with monoclonal antibodies against serotypes 2a, 4, and 15, which are prevalent in Norway. The MICs of benzylpenicillin, sulfasomidine, rifampin, and ofloxacin were determined by an agar dilution method with PDM II Antibiotic Sensitivity Medium (AB Biodisk, Solna, Sweden) supplemented with 5% horse blood. DNA fingerprinting was applied for identification of different clones of *N. meningitidis*. Genomic patterns were obtained by using a modification of the method of Bjorvatn et al. (3). In short, *N. meningitidis* was harvested from blood agar plates and was lysed with lysozyme, EDTA, sarcosyl, and Triton X-100. RNA was removed with RNase, and extractions with phenol-chloroform removed proteins. Electrophoresis was carried out for 20 h on 4% polyacrylamide gel at a setting of 22 mA and 580 V. The bands were visualized with UV light after staining with ethidium bro-

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TABLE 1. Tonsillopharyngeal carriers of *N. meningitidis* before
ofloxacin treatment

Population	No. (%) of strains in:					
	Total	Volunteers	Carriers	Serogroup		
				B	Y	NG[a]
Students	348	343 (99)	83 (24)	21	11	51
Teachers	42	38 (90)	0 (0)	0	0	0
Employees	12	11 (92)	1 (9)	0	0	1
Total	402	392 (98)	84[b] (21)	21	11	52

[a] NG, nongroupable *N. meningitidis*.
[b] Eighty carriers were treated, but five of these were excluded from the analysis of efficacy.

TABLE 2. Tonsillopharyngeal carriage of *N. meningitidis* in 75
evaluable students before and after a single dose of 400 mg of
ofloxacin and in 233 noncarrier students who were not treated but
resampled at day 33

Population and day of study	No. (%) of strains in:				
	Volunteers	Carriers	Serogroup		
			B	Y	NG[a]
Carriers at day −2					
−2 (sampling)	75 (100)	75 (100)	21	7	47
0 (treatment)	75 (100)	Not done			
3 (first control)	75 (100)	0 (0)	0	0	0
7 (second control)	59 (79)	3 (5)	0	0	3[b]
33 (third control)	71 (95)	6 (8)	0	2	4[c]
Noncarriers at day −2					
−2 (Sampling)	233 (100)	0 (0)	0	0	0
33 (Resampling)	233 (100)	6 (3)	4	0	2

[a] NG, Nongroupable *N. meningitidis*.
[b] Strains different from those found before treatment.
[c] Two of these carriers harbored similar clones on days −2 and 33.

mide and were photographed. The prints obtained were compared visually and were grouped into clones or clonal clusters with at least 90% similarity.

After receiving oral and written information about the study and possible adverse effects, the meningococcal carriers gave written consent and received a single dose of 400 mg of ofloxacin (day 0) within 72 h after sampling (day −2). Five individuals were excluded from the study because of the absence of controls or because drug was administered too late. All carriers were asked to report adverse events. No carrier had received antibiotic treatment in the last 2 weeks before screening, and no carrier was pregnant. Because of the epidemic situation, a high level of anxiety prevailed at the college and in the local community. We therefore found a placebo-controlled trial inappropriate. Ethical approval for the study was obtained from the regional research ethics committee. Tonsillopharyngeal samples for culture were obtained on days 3, 7, and 33 after therapy from those who had been treated. At the last sampling time, a total of 352 subjects were screened for meningococcal carriage, and 233 of these were noncarrier students at the first sampling. In order to observe the differences between carriers and noncarriers with respect to contraction of meningococcal carriage, we introduced the concept of carriage aquisition rate (CAR). CAR denotes the number of strains aquired per person per day multiplied by 100. Data for the first 3 days after ofloxacin treatment were excluded from the calculation because of the prolonged half-life of the drug in tissue. Computation of statistical significance was performed by using the Pearson chi-square test, and $P < 0.05$ was chosen as the level of significance.

The results of the first meningococcal sampling (day −2) are summarized in Table 1. Of the 392 subjects examined, 84 (21%) were carriers of *N. meningitidis*. The three students who had previously suffered meningococcal disease were not carriers by this time. The proportion of female carriers was 62% (68% of the students were females). The case strains and 15 carrier strains were serotype 15, while 19 and 1 were isolates of serotypes 4 and 2a, respectively; 49 isolates were nontypeable. Two carriers harbored a sulfasomidine-resistant B:15:P1.7,16 strain, the most prevalent case strain in Norway. Several other strains had patterns similar to those seen in case isolates. DNA fingerprinting of the case and carrier isolates revealed 41 different genome patterns, with 1 to 12 strains having the same pattern. Twenty-one strains had patterns that did not resemble those of the other strains, and one of these belonged to one of the case patients. The two case isolates represented different meningococcal clones, and the number of students whose isolates

showed similar patterns were zero and five, respectively. These five strains were serogroup B or nongroupable, all were serotype 15 or 3, and all were resistant to sulfasomidine. The two case strains were susceptible to benzylpenicillin, rifampin, and ofloxacin but were resistant to sulfasomidine (MIC, ≥16 mg/liter). All 84 carrier isolates were susceptible to benzylpenicillin (MIC, ≤0.03 to 0.25 mg/liter), rifampin (MIC, ≤2 mg/liter), and ofloxacin (MIC, ≤0.03 mg/liter). Sulfasomidine resistance was detected in 17 strains (20%).

Eighty culture-positive individuals were treated with 400 mg of ofloxacin orally as a single dose. Three students reported probable adverse events; all complained of mild dizziness. After 3 days, *N. meningitidis* could not be isolated from any of the 75 carriers from whom samples were obtained (Table 2). Seven days after therapy, all three detected strains had different genomic patterns compared with those of the strains isolated from the same individuals before treatment. At the last sampling time (day 33), *N. meningitidis* was isolated from 6 of 71 attending students. In two of these carriers, who were culture negative on days 3 and 7, we identified the same clone at the first and last samplings. Calculating the CAR for the carrier group among students, we found the following: CAR = [(6/71)/30] × 100 = 0.28 strains per person per day.

On day 33, 13 meningococcal carriers were found among 352 subjects at the college. Resampling of 233 students who were noncarriers on day −2 revealed 6 new meningococcal carriers. CAR for noncarriers was estimated to be [(6/233)/36] × 100 = 0.072 strains per person per day, which was significantly lower than that for the carrier group ($P = 0.026$). The CAR of *N. meningitidis* among students was about four times higher in the carrier group compared with that in noncarriers in the present study. CAR among teachers and other employees was zero. Administration of a single dose of ofloxacin to 80 of 84 meningococcal carriers reduced the carrier rate at the college from 21.4 to 3.7%. During the subsequent 6 months, no new case of meningococcal disease was observed in Øystese.

In the present study, a single dose of 400 mg of ofloxacin proved to be effective in eradicating meningococcal carriage in the throat. The high level of genetic diversity of the strains among carriers of *N. meningitidis* serogroup B found in the

present study is consistent with that found in other trials (4, 8). The CAR of the initially culture-negative students proved to be four times lower than that of meningococcal carriers. This significant difference between carriers and noncarriers may be due to a disposition, either genetic, sociological, or environmental, that was already present prior to treatment. However, eradication of meningococcal carriage may concomitantly affect the ecological balance in the normal throat flora in a way that enhances recolonization from the surroundings. The two meningococcal carriers in the treatment group who were culture negative on days 3 and 7 were colonized with the same strain before treatment and at the last screening. This may be due to sampling failure on days 3 and 7 or ofloxacin suppression of tonsillopharyngeal growth for a week, but most probably they were recolonized. On days 7 and 33, different clones were isolated from three carriers compared with the clones found before treatment. It thus appears that a single dose of ofloxacin eradicated the initial strain in all treated subjects.

Since 1977, household contacts of less than 15 years of age in Norway have been given penicillin orally for 1 week to treat possible incipient meningococcal disease (17). However, such therapy does not eradicate carriage of *N. meningitidis*, partly because of the lack of penetration of penicillin into phagocytic cells. On the other hand, Hart et al. (7) have treated all household contacts and relatives of patients with meningococcal disease with ciprofloxacin, rifampin, or ceftriaxone to eradicate meningococcal carriage. To identify carriers at risk and to selectively eradicate pathogenic *N. meningitidis* from the environment, Kristiansen et al. (9) have recommended tracing of similar strains to the case isolate by DNA fingerprinting and subsequent rifampin therapy for all such carriers. Hence, only carriers with the disease-causing strain could receive chemotherapy, and development of resistant isolates would probably diminish. For general use, however, such strain tracing appears to be too time-consuming and expensive and should be restricted to selected studies. Until reliable and rapid methods for screening of contacts are available, single-dose ofloxacin therapy should be restricted to household contacts.

In the present study, a single dose of ofloxacin eradicated meningococcal carriage in 100% of the volunteers for 1 week. After 33 days, only 2 of 71 (2.8%) attending volunteers seemed to be reinfected, indicating a low eradication failure rate for ofloxacin. No new case of meningococcal disease was observed in the following 6 months. Accordingly, because treatment with ofloxacin effectively reduces the carrier rate in a population, it may prevent the spread of *N. meningitidis* and outbreaks of meningococcal disease.

REFERENCES

1. **Band, J. D., and D. W. Frazer.** 1984. Adverse effect of two rifampicin dosage regimes for the prevention of meningococcal infection. Lancet **i:**101–102.

2. **Beam, W. E., N. R. Newberg, L. F. Devine, W. E. Pierce, and J. A. Davies.** 1971. The effect of rifampicin on the nasopharyngeal carriage of *Neisseria meningitidis* in a military population. J. Infect. Dis. **124:**39–46.

3. **Bjorvatn, B., V. Lund, B.-E. Kristiansen, L. Korsnes, O. Spanne, and B. Lindquist.** 1984. Applications of restriction endonuclease fingerprinting of chromosomal DNA of *Neisseria meningitidis*. J. Clin. Microbiol. **19:**763–765.

4. **Caugant, D. A., E. A. Høiby, E. Rosenqvist, L. O. Frøholm, and R. K. Selander.** 1992. Transmission of *Neisseria meningitidis* among asymptomatic military recruits and antibody analysis. Epidemiol. Infect. **109:**241–253.

5. **Dworzack, D. L., C. C. Sanders, E. A. Horowitz, J. M. Allais, M. Sookpranee, W. E. Sanders, Jr., and F. M. Ferraro.** 1988. Evaluation of single-dose ciprofloxacin in the eradication of *Neisseria meningitidis* from nasopharyngeal carriers. Antimicrob. Agents Chemother. **32:**1740–1741.

6. **Gaunt, P. N., and P. E. Lambert.** 1988. Single-dose ciprofloxacin for the eradication of pharyngeal carriage of *Neisseria meningitidis*. J. Antimicrob. Chemother. **21:**489–496.

7. **Hart, C. A., L. E. Cuevas, P. Kazembe, G. K. Mughogho, and G. S. Tillotson.** 1992. The use of ciprofloxacin to eradicate oropharyngeal carriage of *Neisseria meningitidis*: a preliminary analysis. Adv. Antimicrob. Antineopl. Chemother. **11(2):**167–171.

8. **Kristiansen, B.-E., B. Bjorvatn, V. Lund, B. Lindquist, and E. Holten.** 1984. Differentiation of B15 strains of *Neisseria meningitidis* by DNA restriction endonuclease fingerprinting. J. Infect. Dis. **150:**672–677.

9. **Kristiansen, B.-E., Y. Tveten, E. Ask, T. Reiten, A.-B. Knapskog, J. Steen-Johnsen, and G. Hopen.** 1992. Preventing secondary cases of meningococcal disease by identifying and eradicating disease-causing strains in close contacts of patients. Scand. J. Infect. Dis. **24:**165–173.

10. **Lystad, A., and S. Aasen.** 1991. The epidemiology of meningococcal disease in Norway 1975–91. Natl. Inst. Public Health Ann. **14:**57–65.

11. **Metz, R., K. G. Naber, K. Hönneknovel, T. Lohn, and F. Sörgel.** 1990. Comparative pharmacokinetics and body fluid penetration of ofloxacin and ciprofloxacin in man. Proc. 3rd Int. Symp. New Quinolones. Eur. Clin. Microbiol. Infect. Dis. **1990:**30–31.

12. **Perea, E. J.** 1990. Ofloxacin concentrations in tissues involved in respiratory tract infections. J. Antimicrob. Chemother. **26(Suppl. D):**55–60.

13. **Pugsley, P. M., D. L. Dworzack, E. A. Horowitz, T. A. Cuevas, W. E. Sanders, Jr., and C. C. Sanders.** 1987. Efficiency of ciprofloxacin in the treatment of nasopharyngeal carriers of *Neisseria meningitidis*. J. Infect. Dis. **156:**211–213.

14. **Renkonen, O. V., A. Sivonen, and R. Visakorpi.** 1987. Effect of ciprofloxacin on carrier rate of *Neisseria meningitidis* in army recruits in Finland. Antimicrob. Agents Chemother. **31:**962–963.

15. **Rosenquist, E., E. Wedege, E. A. Høiby, and L. O. Frøholm.** 1990. Serogroup determination of *Neisseria meningitidis* by whole-cell ELISA, dot-blotting and agglutination. Acta Pathol. Microbiol. Scand. **98:**501–506.

16. **Van Landuyt, H. W., B. Gordts, and G. D. Hondt.** 1988. Pharmacokinetic evaluation of ofloxacin in serum and tonsils. J. Antimicrob. Chemother. **22(Suppl. C):**81–83.

17. **Wall, R. A.** 1988. The chemoprophylaxis of meningococcal infection. Antimicrob. Chemother. **21:**698–700. (Editorial.)

18. **Wedege, E., E. A. Høiby, E. Rosenquist, and L. O. Frøholm.** 1990. Serotyping and subtyping of Neisseria meningitidis isolates by co-agglutination, dot-blotting and ELISA. J. Med. Microbiol. **31:**195–201.