J KAU: Med. Sci., Vol 7, No. 2, pp. 81-86 (1419 A.H. / 1999 A.D.)

Antibiotic Susceptibility of *Neisseria meningitidis* Strains Isolated in Makkah, Saudi Arabia

SOLIMAN M. EL-SHEIKH, PhD
Department of Medical Microbiology, Faculty of Medicine & Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT. The antimicrobial activity of 10 antibiotics against 42 Group A *Neisseria meningitidis* strains beta-lactamase non-producing was determined using the disc diffusion method and dilution technique. All isolates were susceptible by the DDM to penicillin, ampicillin, cefuroxime, ceftriaxone, cefotaxime, chloramphenicol ciprofloxacin, and rifampicin. Thirty-two (76%) were resistant to sulphamethoxazole and trimethoprim/sulphamethoxazole. MICs of the isolates showed that ceftriaxone, cefuroxime, and cefotaxime had greater inhibitory activities than penicillin or rifampicin.

Keywords: *Neisseria meningitidis*, Antibiotic susceptibilities.

Introduction

Meningococcal meningitis continues to pose a serious threat to the population of the "meningitis belt" of sub-Sahara Africa. In this region, which extends across Africa from Sudan in the east to the Gambia in the West, major epidemics of meningococcal disease occur every few years[1]. These epidemics are caused predominantly by *Meningococci* belonging to serogroup A. Ironically, the 1987 epidemic in this "meningitis belt" originated from Saudi Arabia with a strain introduced by southeast Asians who were performing pilgrimage (Hajj)[2]. The particular strain responsible for this outbreak was later designated as "clone III-I"[2]. Antimicrobial therapy should be promptly initiated in all patients with suspected meningococcal disease because of the rapidity with which patients may deteriorate. Although the use of penicillin has greatly de-

Correspondence & reprint requests to: Dr. Soliman M. El-Sheikh, P.O. Box 9029, Jeddah 21413, Saudi Arabia.
Accepted for publication: 8 June 1998. Received: 31 March 1998.

creased the mortality rate due to meningococcal infections, its usefulness is now being compromised[3]. In 1985 the first clinical isolate of *Neisseria meningitidis* relatively resistant to penicillin (N men RRP), defined as having a MIC of penicillin of 0.1-1.0 μg/ml, was identified in Spain[4]. Since then, N men RRP has been reported from other countries including South Africa[5] and Canada[6]. However, the emergence of penicillin-resistant *Meningococci* and the toxicity of chloramphenicol make it necessary to search for a more suitable antimicrobial agent. Barson *et al*[7] reported that ceftriaxone is highly active against *Meningococci* and attains more rapid sterilization of the cerebrospinal fluid (CSF) than do ampicillin and chloramphenicol[7]. Of the various antibiotics that have been used to eradicate the carriage of *Meningococci*, rifampicin is recommended for prophylaxis. However, rifampicin-resistant *Meningococci* have been reported[8]. The purpose of the present study was to determine the *in-vitro* activity of penicillin and 9 other antimicrobial agents against 42 clinical isolates of *Meningococci* isolated from patients admitted to the King Abdulaziz Hospital in Makkah during the 1994 pilgrimage (Hajj) season.

Patients and Methods

Bacterial Strains: Forty-two (42) isolates of *Neisseria meningitidis* from CSF from patients admitted to the King Abdulaziz Hospital in Makkah during pilgrimage (Hajj) 1994 were studied. Identification of *Meningococci* was based on morphology, Gram's stain, oxidase reaction, and fermentation of glucose and maltose. Serogroups were determined by slide agglutination with polyclonal antisera to serogroups A, B, C, W135, X, Y, and Z (Wellcome Diagnostics). Two penicillin-resistant strains from Enid Sutcliffe (Public Health Laboratory, Withington Hospital, Manchester, England). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as control strains for susceptibility tests.

β-Lactamase Test: Commercially available β-lactamase (Nitrocefin sticks) Unipath (Oxoid, U.K.) were used for the detection of β-lactamase production, as directed by the manufacturer.

Antibiotics: The antibiotic standard powders tested and their suppliers are as follows: penicillin G and cefuroxime (Glaxo Laboratories, Ltd., Greenford, England); cefotaxime (Roussel, Montreal, Canada); ceftriaxone (Roche Products, Ltd., England); and rifampicin (Ciba-Geigy, Basal, Switzerland). Antibiotic discs used were: penicillin G (0.6 ug), ampicillin (2 ug), chloramphenicol (10 ug), ceftriaxone (30 ug), cefuroxime (30 ug), cefotaxime (30 ug), rifampicin (5 ug), ciprofloxacin (1 ug), sulphamethoxazole (25 ug), and trimethoprim/sulphamethoxazole (25 ug) and all were obtained from Oxoid, England.

Media: Each strain was grown on chocolate blood agar and incubated at 37°C in a humidified incubator with 5%(v/v) CO_2 for 24h. Muellar-Hinton broth of the same batch was used for all investigations.

Antimicrobial Susceptibility Tests: Disc diffusion was performed by the Stokes comparative agar diffusion method[9] using Muellar Hinton agar. Strains were resistant when the zone diameter of the tested organism was at least 3 mm less than the zone produced by the control and were considered sensitive if greater. Minimal Inhibitory Concentrations (MICs) was determined by the broth method (NCCLS, 1993)[10] and tested organisms were diluted to 0.5 McFarland standard (approximately $1.5x10^8$ cfu/ml) and further diluted 1:10. The final inoculum on the agar plate will then be approximately 10^4 cfu/ml. The MIC was defined as the lowest concentration of antibiotics that allow no visible growth.

Results

Forty-two (42) *Neisseria meningitidis* strains isolated from the CSF of patients with meningitis admitted to the King Abdulaziz Hospital in Makkah during pilgrimage (Hajj) in 1994 were studied. All isolates were group A, β-lactamase non-producing and susceptible by the DDM to penicillin, ampicillin, cefuroxime, ceftriaxone, cefotaxime, chloramphenicol, ciprofloxacin, and rifampicin (76%) were resistant to sulphamethoxazole and trimethoprim/sulphamethoxazole.

MICs of the isolates showed that ceftriaxone, cefuroxime, and cefotaxime had greater inhibitory activities than penicillin or rifampicin (Table 1.).

TABLE 1. MICs of 42 strains of *neisseria* meningitidis to some β-lactam and rifampicin.

Antibiotic	MIC Range (ug/ml)	MIC 50 (ug/ml)	MIC 90 (ug/ml)
Ceftriaxone	≤ 0.0005 - 0.002	0.001	0.002
Cefotaxime	≤ 0.0005 - 0.002	0.001	0.002
Cefuroxime	≤ 0.0005 - 0.004	0.002	0.002
Penicillin	0.016 - 0.032	0.016	0.032
Rifampicin	0.008 - 0.064	0.032	0.064

Discussion

Meningococci are sensitive *in-vitro* to a wide range of antibiotics but are frequently partially or completely resistant to sulfonamides[11]. Mikhail *et al*[12] reported on the isolation of sulfadiazine and trimethoprim/sulphamethoxazole resistant meningococci in Egypt and state that 74% of all the *Neisseria meningitidis* isolates from patients and controls in Cairo were resistant to sulfadiazine and trimethoprim/sulphamethoxazole. This is in broad agreement with our own results of 76% resistance to sulphamethoxazole and trimethoprim/sulphamethoxazole. Although *Meningococci* are sensitive to many antibiotics, none is as effective as sulfadiazine in eradicating the nasopharyngeal carriage of *Meningococci*. Penicillin is commonly used for the treatment

of meningococcal infection (all of the 42 strains in the present study tested for sensitivity to penicillin were sensitive to 0.032 mg/L). Meningococcal infections are usually treated with ampicillin, penicillin, or a combination of penicillin and chloramphenicol. However, the emergency of penicillin-resistant *Meningococci* and the toxicity of chloramphenicol makes it necessary to search for a more suitable antimicrobial agent. Considering that *Meningococci* have a tendency to develop resistance to rifampicin, and in view of the failure of rifampicin to prevent secondary cases as well as the ability of rifampicin-resistant *Meningococci* to cause systemic disease[8], an alternative to rifampicin would be of value. Minocycline and ciprofloxacin are not suitable for use in young children or in pregnancy. The present study shows *Neisseria meningitidis* to be extremely sensitive to ceftriaxone which had greater minimal inhibitory activity than penicillin or rifampicin. Schaad *et al*[13] reported that ceftriaxone is effective in eradicating the nasopharyngeal carriage of *Meningococci*. Thus, ceftriaxone should be considered for therapy or prophylaxis in an outbreak of meningococcal disease. Intravenous penicillin-G remains the drug of choice. In the study of Barlas *et al*[14], penicillin-G was given at 4 mega units every four hours to all patients. Ceftriaxone is an alternative choice as it can be given in a single intramuscular dose; thus, it facilitates nursing care and certainly results in financial savings[15]. A case of great concern is that description of beta-lactamase producing strains of *Neisseria meningitidis* causing clinical disease in South Africa[5] and Spain[16]. Although they appear to be local isolates, their potential dissemination is a matter of serious concern. Microbiologists need to be aware that penicillin-resistant *Meningococci* can appear spontaneously and that *in-vitro* susceptibility studies with MIC determinations as well as tests of β-lactamase production, should be performed on all clinical isolates of *Neisseria meningitidis*.

Acknowledgment

We thank Professor A. O. Osoba, Consultant Microbiologist, King Khalid National Guard Hospital, Jeddah for critical reviewing of the manuscript.

References

[1] **Lapeyssonie L.** La meningite cerebrospinale en Afrique. *Bull Who* 1963; 28: 3-114.

[2] **Moore PS, Reeves MW, Schwartz B, Gellin BG, Broome CV.** International spread of an epidemic group A *Neisseria meningitidis* strain. *Lancet* 1989; 2: 260-263.

[3] **Saez-Nieto JA, Vasques JA, Marcos C.** *Meningococci* moderately resistant to penicillin [letter]. *Lancet* 1990; 336: 54.

[4] **Saez-Nieto JA, Fontanals D, Garcia-de-Jalon J, Martinez-de-Artola V, Pena P, Morera MA, Verdaguer R, Sanfeliu I, Belio-Blasco C, Perez-Saenz JL.** Isolation of *Neisseria meningitidis* strains with increase of penicillin minimal inhibitory concentrations. *Epidemiol Infect* 1987; 99(2): 463-469.

[5] **Botha P.** Penicillin-resistant *Neisseria meningitidis* in southern Africa [letter]. *Lancet* 1988; 1: 54.

[6] **Riley G, Brown S, Krishnan C.** Penicillin resistance in *Neisseria meningitidis* [letter]. *Lancet* 1990; 324: 997.

[7] **Barson WJ, Miller MA, Brady MT, Powell DA.** Prospective comparative trial of ceftriaxone vs. conventional therapy for treatment of bacterial meningitis in children. *Pediatr Infect Dis* 1985; 4: 362-368.

[8] **Yagupsky P, Ashkenazi S, Block C.** Rifampicin-resistant *Meningococci* causing invasive disease and failure of chemoprophylaxis. *Lancet* 1993; 341: 1152-1153.

[9] **Collins CH, Lyne PM, Grange JM.** *Collins and Lyne's Microbiological Methods, 6th Edition.* Ox-

ford: Butterworth-Heinemann, 1991: 101-168.

[10] **National Committee for Clinical Laboratory Standards.** Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, PA, 1993.

[11] **Fallon RJ.** Meningococcal infections in Scotland, 1977. *Communicable Diseases Scotland, Weekly Report* 1978; 78(9): 7-12.

[12] **Mikhail IA, Girgis NI, Farid Z, Haberberger RL, Woody JN.** Antibiotic susceptibility of *Neisseria meningitidis* in Egypt. *Trop Geograph Med* 1998; **41**: 285.

[13] **Schaad UB, Suter S, Gianella-Borradori A, Pfenninger J, Auckenthaler R, Bernath O, Chesaux JJ, Wedgwood J.** A comparison of ceftriaxone and cefuroxime for the treatment of bacterial meningitis in children. *N Engl J Med* 1990; 322(3): 141-147.

[14] **Barlas S, Safdar MU, Chaudhry AS, Ahamad T, Hashmi IH.** Meningococcal disease: clinical profile of 99 patients. *Ann Saudi Med* 1993; **13**: 237-241.

[15] **Girgis NI, Abu El Ella AH, Farid Z, Woody JN, Lissner C.** Ceftriaxone compared with combination of ampicillin and chloramphenicol in the treatment of bacterial meningitis in adults. *Drugs Expl Clin Res* 1987; **8**: 497-500.

[16] **Fontanals D, Pineda V, Pons I, Rojo JC.** Penicillin-resistant beta-lactamase producing *Neisseria meningitidis* in Spain. *Eur J Clin Microbiol* 1989; **8**: 90-91.

حساسية جرثومة المكورة السحائية المفصولة في مكة للمضادات الحيوية

سليمان الشيخ
قسم الكائنات الدقيقة، كلية الطب والعلوم الطبية، جامعة الملك عبدالعزيز، جدة، المملكة العربية السعودية

المستخلص. لقد جرى تحديد قابلية وإستجابة ٤٢ مكورة سحائية مجموعة (أ) والمعزولة في مكة لعشر مضادات حيوية بطريقة انتشار القرص وطريقة التركيز الأدنى المثبط للنمو الجرثومي. وقد أبدت كل الذريات بطريقة إنتشار القرص استجابة كاملة لكل من البنسلين، أمبيسلين، سيفوركسيم، سفتريكسون، سيفوتكسيم، كلورمفنكول، سبروفلكساسين، والريفمبيسين. وقد أبدت ٢٣ ذرية (٧٦٪) مقاومة لكل من سلفاميثكسازول وترايمثبريم / سلفا ميثكسازول. كما أظهرت طريقة التركيز الأدنى المثبط للنمو الجرثومي أن كلاً من سفرتريكسون، سيفوركسيم وسيفوتكسيم لها تأثير مثبط أكثر من البنسلين والريفمبيسين.