

Susceptibility of *Neisseria meningitidis* Strains from the Civilian Population to Sulfadiazine, Penicillin, and Rifampin

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The minimal inhibitory concentration (MIC) values of sulfadiazine, penicillin, and rifampin for meningococcal strains isolated from civilians during 1970 were compared. The strains were isolated from various sources and geographical areas and represented several serogroups. The ranges of MIC values were as follows: 0.05 to 20 mg/100 ml for sulfadiazine, 0.01 to 0.4 $\mu\text{g}/\text{ml}$ for penicillin, and 0.01 to 0.8 $\mu\text{g}/\text{ml}$ for rifampin. There was no significant relationship between MIC values of sensitive or resistant sulfadiazine strains and the MIC values to the other two antimicrobial agents. Comparisons of sulfadiazine MIC values with inhibition zones around sulfathiazole discs showed excellent correlation, provided the strains were separated into sensitive and resistant groups on the basis of growth at 1 mg/100 ml. Regression curves for penicillin and rifampin sensitivity showed homologous sensitive populations with the strains studied.

After the report by Millar et al. (13) of sulfadiazine-resistant meningococci in a military population, a surveillance of sulfadiazine susceptibility of *Neisseria meningitidis* strains isolated from the civilian population was begun by the Bacterial Immunology Unit, Center for Disease Control (CDC; 4). Later, as the use of penicillin for the treatment of meningococcal disease increased, this laboratory began monitoring *N. meningitidis* strains for their sensitivity to penicillin.

Recent reports (6; L. F. Devine et al., J. Amer. Med. Ass., *in press*) have suggested that rifampin may be useful as a prophylactic agent in the treatment of carriers of meningococci. These workers have used rifampin to treat carriers in military and civilian groups. Devine and Hagerman (7) have established the *in vitro* sensitivities to rifampin of meningococci strains from healthy military carriers. It is important that a base line of sensitivity to rifampin be established for meningococcal strains isolated from the civilian population and that these findings be related to the sensitivity of these same strains to sulfadiazine and penicillin. Results of sensitivity studies of *N. meningitidis* strains to these three antimicrobial agents, sulfadiazine, penicillin, and rifampin, determined by an agar dilution method, are presented here. In addition to the minimal inhibitory concentration (MIC) values, growth inhibi-

tory zone sizes around sulfathiazole, penicillin, and rifampin discs are reported.

MATERIALS AND METHODS

Strains. Meningococcal strains isolated during 1970 from persons in the civilian population of the United States were included in comparisons of susceptibility to sulfadiazine, penicillin, and rifampin. These strains consisted of 289 cultures of the following serogroups and sources: from blood and spinal fluid, 1 group A, 66 group B, 125 group C, 12 group Y, 2 group Z, 1 strain which reacts in two or more antisera (cross-reacting), and 1 rough strain; from pharynx, 16 group B, 6 group C, 4 group X, 2 group Y, 3 group Z, 8 rough, 7 cross-reacting strains, and 1 smooth nonreactive strain; from other sources including eye, sputum, vagina, urethra, urine, and autopsy, 6 group B, 7 group C, and 1 cross-reacting; strains from sources which are not known, 2 group B, 17 group C, and 1 cross-reacting. These strains came from all eight geographical areas of the United States (4).

In addition to 197 of the above strains, nine strains from foreign countries (eight group A strains from Africa and one group C from Canada) and 20 strains from a military installation (all group C) were included in the comparison of end points by the agar dilution method to zone sizes in the disc procedure. Sensitivity studies were also performed on 24 *N. lactamicus* strains isolated during 1970 from healthy carriers.

Approximately half of the *N. meningitidis* strains were submitted to CDC for either identification or

TABLE 1. Susceptibility to sulfadiazine and rifampin of *Neisseria meningitidis* strains isolated from the civilian population

Source	Rifampin MIC ($\mu\text{g/ml}$)	No. of sulfadiazine-sensitive strains in serogroup										No. of sulfadiazine-resistant strains in serogroup				
		B	C	Y	Crosses	Rough	Z	X	A	Smooth	Total	B	C	Y	Crosses	Total
Spinal fluid or blood	0.01	14	6	6		1					27	16	50		1	67
	0.05	20	3	4			1		1		29	5	55			60
	0.1	7	4				1				12	1	6	1		8
	0.2	2	1								3					
	0.4	1									1					
	0.8			1							1					
Pharyngeal	0.01	2				2	1			1	6	2	1		1	4
	0.05	3	1	1	2	3	2	3			15		4			4
	0.1	7		1	4	3		1			16	1				1
	0.2	1									1					
Other	0.01		1								1		1			1
	0.05	4	1		1						6	1	4			5
	0.1															
	0.2	1									1					
Unknown	0.01	1									1		12		1	13
	0.05	1									1		3			3
	0.1												1			1
	0.2		1								1					
Totals		64	18	13	7	9	5	4	1	1	122	26	137	1	3	167

confirmation as well as sensitivity studies. These strains were identified as previously described (10, 11). The remaining strains were checked by a Gram stain, oxidase reaction, and serological grouping with *N. meningitidis* antisera.

Antimicrobial testing. A previously described (11) plate-dilution method was employed. The inoculum was standardized spectrophotometrically to a density equal to a McFarland no. 5 and streaked with a 1-mm loop onto the plates containing a series of antimicrobial concentrations in Mueller-Hinton agar (MHA). The term MIC was applied to the concentration of antimicrobial agent which showed marked reduction of bacterial growth as compared to the confluent growth on a control plate containing only MHA. If a slight haze of growth or a single colony extended for several plates (contamination eliminated), the MIC was recorded within one plate dilution of the last concentration on which any growth appeared. The method used for disc-sensitivity studies was that described by Bennett et al. (3) with two modifications: the inoculum used for the plate-dilution method was diluted with Mueller-Hinton broth to the recommended optical density, and discs were applied to a seeded plate (15 by 150 mm) containing 75 ml of MHA.

Antimicrobial agents. Sulfadiazine dilutions were prepared from sodium sulfadiazine injectable containing 0.25 g/ml. Penicillin dilutions were made from potassium penicillin which contained 1,600 units/mg. Dilutions of both of these antimicrobials

were prepared with sterile distilled water. Rifampin (Mann Research Laboratories) was dissolved in *N,N* dimethyl formamide (14) and diluted in distilled water. The discs employed were 300- μg sulfathiazole, 10-unit penicillin, and 30- μg rifampin from commercial sources.

RESULTS

Rifampin (Mann) used in this study was only partially soluble in ethanol; however, this lot was completely soluble in dimethylsulfoxide (DMSO) and in *N,N* dimethyl formamide. A sample of ethanol-soluble rifampin (Pittman-Moore) was compared to the Mann rifampin sample by using pyrolysis gas-liquid chromatography and ultraviolet (UV), visible, and infrared spectra absorption techniques. The two samples differed significantly in their UV and visible absorption characteristics. The solvent used for the spectral studies was *N,N* dimethyl formamide. The ethanol-soluble sample had maximum absorptions at 340.5 and 481.5 nm on the Cary 14 recording spectrophotometer, whereas the Mann sample showed maximum absorptions at approximately 341.5 and 482.5 nm (assays performed by Judy Hicks, Biophysical Separations Unit, CDC). A comparison by the plate-dilution method of the Pittman-Moore rifampin sample dissolved in ethanol and the Mann rifampin sample dissolved in DMSO

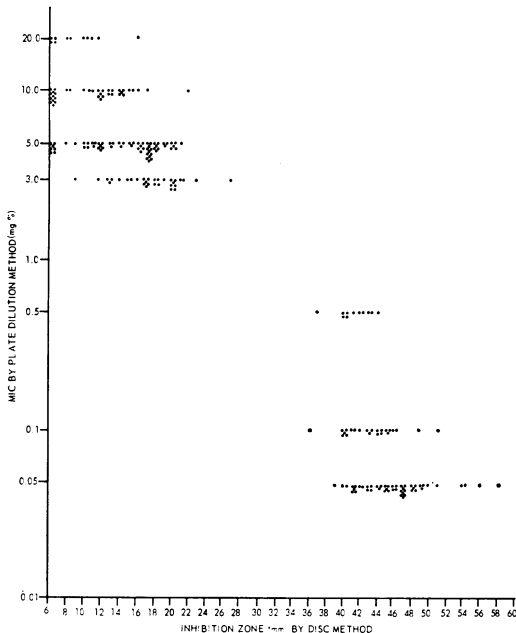


FIG. 1. Relationship between plate-dilution sensitivity to sulfadiazine and inhibition zone around 300-µg sulfathiazole disc.

or *N,N* dimethyl formamide showed complete correlation of MIC values with 16 test strains of *N. meningitidis*. No inhibition of growth of the 16 test strains was demonstrated when these solvents were diluted to concentrations employed in the test.

Meningococcal strains classified by serogroup and source are shown in Table 1 with their susceptibilities to sulfadiazine and rifampin as determined by the plate-dilution method. Strains were recorded as sensitive to sulfadiazine if inhibited by 1 mg/100 ml or less, and strains growing at ≥ 1 mg/100 ml of sulfadiazine were considered to be resistant. The MIC values of rifampin for sulfadiazine-sensitive and -resistant strains ranged from ≤ 0.01 to 0.8 µg/ml. Of 132 sulfadiazine-sensitive strains, 28.7% failed to grow at 0.01 µg/ml, 41.8% had MIC values of 0.05 µg/ml, 23% had MIC values of 0.1 µg/ml, and 6.5% had MIC values of 0.2 to 0.8 µg/ml. The MIC values of rifampin for 167 sulfadiazine-resistant strains showed that fewer strains grew at the higher dilutions with 41.8, 42.8, 13.2, and 2% for rifampin concentrations of 0.01, 0.05, 0.1, and ≥ 0.2 µg/ml, respectively. The MIC values of rifampin for strains of different serogroups of meningococci varied only slightly. Of 90 group B strains, 94.4% had rifampin MIC values of ≤ 0.1 µg/ml; 98.7% of 155 group C strains, and 92.8%

of 14 group Y strains had the same range of MIC values. The classification "other strains" was composed of several serogroups plus some rough and untypable strains. All of these strains had MIC values of ≤ 0.1 µg/ml. Penicillin MIC values of all the strains in Table 1 were ≤ 0.4 µg/ml.

MIC values of 24 *N. lactamicus* strains were determined. All of these strains were sensitive to sulfadiazine with MIC values ranging from 0.05 to 0.5 mg/100 ml, and had penicillin MIC values of ≤ 0.05 µg/ml. Only 16.6% of these *N. lactamicus* strains had rifampin MIC values of ≤ 0.1 µg/ml, whereas 83.4% had MIC values of ≥ 0.2 µg/ml.

In Fig. 1, the sulfadiazine MIC values of 225 strains are compared with zone sizes around a 300-µg sulfathiazole disc. The data for both procedures are from a single determination with each test. All strains with MIC values of ≥ 3.0 mg/100 ml had zone sizes of ≤ 27 mm. Zone sizes of ≥ 40 mm were obtained with all but three of the strains with MIC values of ≤ 0.5 mg/100 ml. The MIC values and zone sizes for these strains were as follows: 0.5 mg/100 ml, 37 mm; 0.1 mg/100 ml, 36 mm; and 0.05 mg/100 ml, 39 mm. When the procedure was repeated three times with these three strains and the averages were determined, the MIC values remained un-

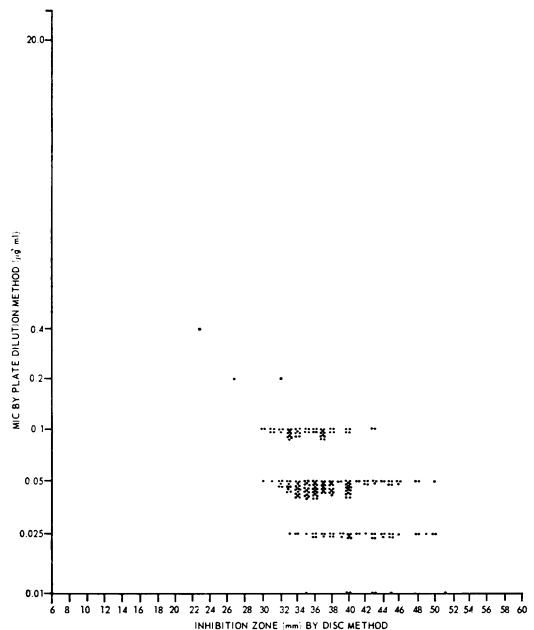


FIG. 2. Relationship between plate-dilution sensitivity to penicillin and inhibition around 10-unit penicillin disc.

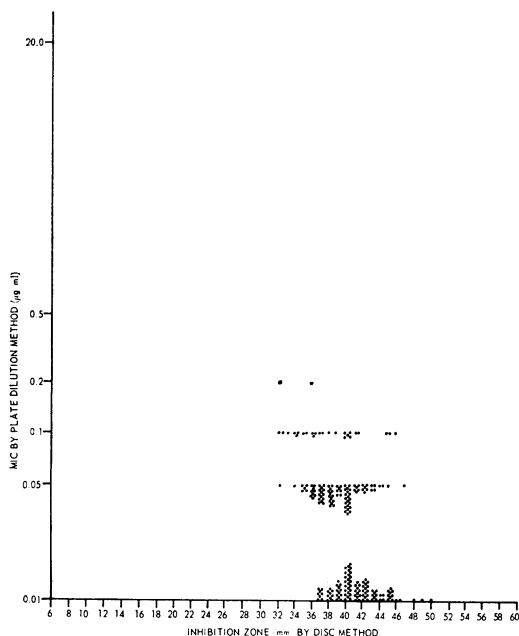


FIG. 3. Relationship between plate-dilution sensitivity to rifampin and inhibition zone around 30- μ g rifampin disc.

changed for all three, but the zone sizes were 38, 38, and 41 mm, respectively.

The penicillin MIC values of the same 225 strains are compared with zone sizes around 10-unit penicillin discs in Fig. 2. The zone sizes ranged from 23 to 51 mm with MIC values of 0.01 to 0.4 μ g/ml. Rifampin-sensitivity results for these strains are shown in Fig. 3. The zone sizes ranged from 32 to 50 mm for MIC values of 0.01 to 0.2 μ g/ml.

DISCUSSION

Several workers (5, 7, 15) dissolved rifampin in ethanol or methanol for their sensitivity studies. The lot of rifampin used in this study was only partially soluble in ethanol; however, this lot was completely soluble in DMSO and in *N,N* dimethyl formamide which were used as solvents by other workers (6, 12, 14). A sample of ethanol-soluble rifampin (Pittman-Moore) was supplied by Dieter Stottmeier. When the two samples were compared, differences were found in their solubility and ultraviolet and visible absorption characteristics; however, there appeared to be no significant difference in the antimicrobial activity of the two samples when tested with strains of *N. meningitidis*.

Sensitivity studies on 289 strains isolated from the civilian population during 1970 resulted in rifampin MIC values which showed no signifi-

cant correlation to the MIC values of sulfadiazine or penicillin. The rifampin MIC values for strains from a variety of sources and of different serogroups ranged from ≤ 0.01 to 0.8 μ g/ml. These values are well below the levels of rifampin normally obtained in the blood after a variety of treatment schedules (5; Devine et al., *J. Amer. Med. Ass.*, *in press*). Since rifampin has been suggested primarily for prophylactic use in managing the meningococcal carrier, the model of Devine et al. (8) should be considered. These workers suggested that a drug must be secreted in the saliva at a concentration approaching the MIC value of reference strains as determined *in vitro* by the plate-dilution method for the antimicrobial agent to be effective in eliminating the strain from the nasopharynx. Devine et al. (*J. Amer. Med. Ass.*, *in press*) reported saliva levels of >0.125 μ g/ml 3.5 hr after three daily doses of 600 mg of rifampin. When these workers used rifampin in the prophylactic treatment of meningococcal carriers, the carrier rate was reduced 86% during treatment and 79% at 11 days after completion of treatment. MIC values of reference strains they studied were lower than levels of rifampin which could be demonstrated in saliva of treated carriers. Deal and Sanders (6) reported the elimination of meningococci from carriers with strains having MIC values of 0.062 to 1.0 μ g/ml. Of the 289 meningococcal strains included in this study, the MIC values of 281 strains (97.2%) was 0.1 μ g/ml or less and for the remaining eight strains was less than 1.0 μ g/ml. These results suggest that most of these strains could be eliminated from carriers by rifampin treatment. However, Devine et al. (*J. Amer. Med. Ass.*, *in press*) reported rifampin-resistant strains isolated from 4 of 93 carriers after rifampin treatment. Prior to treatment, the MIC values of the meningococcal strains of all the carriers was ≤ 0.125 μ g/ml. These authors as well as others (5, 6) have warned that rifampin should not be used in epidemiological situations until the survival rate of rifampin-resistant meningococci is established. We concur with this position.

Comparisons of sulfadiazine MIC values of 222 of 225 strains of meningococci with the zone sizes around sulfathiazole discs show complete correlation in interpretation of sensitivity when the regression curves established by Bennett et al. (3) were employed. The three remaining strains with sensitive MIC values had zone sizes that fell into the intermediate range. Bennett et al. (3) stated that the probability of encountering strains in this range was in the area of one or two in 270 determinations. Since they used a geometric mean of three MIC values and compared these to an arithmetic average of three zone sizes, it was

significant that in the present study, where a single MIC determination was compared in a regression curve with a single zone-size determination, the same correlation between these values was found with 99% of the strains, as had previously been reported (3). Since the agreement between these two methods is excellent for separating meningococcal strains into sulfadiazine-resistant or -sensitive groups, laboratories which do not perform the plate-dilution method could use the disc-sensitivity method for testing susceptibility of meningococcal isolates to sulfadiazine. However, we recommend that strains with zone sizes within the intermediate range (37 to 39 mm) be again tested by the agar plate-dilution method for MIC determinations.

It is important to note that these data only supported the correlation between the two procedures when used to separate meningococci into either resistant or sensitive populations. When a given zone size is produced by a strain, one can only determine from this regression curve whether the MIC would be in the <1 mg/100 ml or >1 mg/100 ml range; interpolation of the actual MIC is not possible.

If the MIC to penicillin or zone sizes around penicillin discs for the strains of meningococci studied are recorded on the ordinate of a graph with the percentage of cultures arranged on the abscissa, the resulting curve would show a monophasic distribution. According to Bauer (2), this type of curve is indicative of a homogeneous population. Since Dowd et al. (9) showed that penicillin was unsatisfactory for prophylactic treatment of carriers, penicillin is recommended only for treatment of meningococcal disease. For this reason, the maximum level obtainable in the blood and spinal fluid should be considered in determining the *in vitro* concentration required to separate penicillin-resistant and -sensitive strains. The penicillin concentration used by Bauer (1) in an agar method to separate resistant and sensitive organisms was 5 units/ml. Since no strain was encountered with an MIC greater than levels that can be obtained *in vivo* and no strain tolerated a concentration of antibiotic appreciably higher than that which inhibited the majority of meningococcal strains studied, the population appears to be sensitive by the two concepts usually applied to susceptibility studies as discussed by Bauer (2). Therefore, the regression curves in this study would indicate that meningococcal strains with zone sizes of 23 mm or greater around 10-unit penicillin discs would represent penicillin-sensitive strains.

The same type of monophasic curve described for MIC values of penicillin with the meningococcal strains studied would result if MIC values

of rifampin or zone sizes around rifampin discs were applied to the same type of graph. The strains in this study would all appear to be in a homogeneous sensitive population; therefore, strains with zone sizes of ≥ 32 mm would represent rifampin-sensitive meningococci. Devine et al. (J. Amer. Med. Ass., *in press*) reported rifampin-resistant meningococcal strains with MIC values of ≥ 64 $\mu\text{g/ml}$ isolated from treated carriers.

Subsequent to the completion of this study, 10 rifampin-resistant strains were obtained from L. F. Devine. These strains consisted of the following serogroups: two 29E, two C, one group RAS 10, two group RAS', two group B, and one group Bo. These strains were reported to be representative of the rifampin-resistant meningococci strains encountered by this group (Devine et al., J. Amer. Med. Ass., *in press*). All of these strains grew at 128 μg of rifampin per ml and had no inhibition zones around 30- μg rifampin discs. These data would indicate that either a plate dilution or disc method could be used to distinguish between rifampin-sensitive and -resistant strains.

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