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Comparison of In Vitro Susceptibility of *Neisseria gonorrhoeae* to Trimethoprim-Sulfamethoxazole on Three Different Media

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In vitro susceptibility of 100 clinical isolates of *Neisseria gonorrhoeae* to trimethoprim-sulfamethoxazole (TMP-SMZ combination; 1:19) was determined by the agar dilution technique using three different media under similar conditions. On Oxoid diagnostic sensitivity test, Mueller-Hinton, and GC agar media, the percentage of isolates inhibited by 2.5 μg or less of TMP per ml and 47.5 μg or less of SMZ per ml were 95, 90, and 84%, respectively. TMP-SMZ appeared to be effective in vitro against *N. gonorrhoeae* despite differences in the types of media used.

Gonorrhea is presently a major infectious disease problem. The increased frequency of penicillin-resistant strains of *Neisseria gonorrhoeae* in uncomplicated infection (9, 16, 19) has made it imperative that clinicians seek other effective chemotherapeutic agents. Although tetracycline is effective in eradicating anogenital gonorrhea, resistance to this antibiotic is becoming increasingly apparent (2, 12). Similarly, spectinomycin-resistant strains of *N. gonorrhoeae* have been isolated (12, 17). Moreover, cross-resistance to multiple antibiotics, including penicillin, tetracycline, spectinomycin, chloramphenicol, and erythromycin, is now being reported (10, 12, 15).

Trimethoprim (2,4-diamino-5[3,4,5-trimethoxybenzyl]pyrimidine; TMP) in combination with a sulfonamide has demonstrated in vitro synergism in inhibiting growth of a variety of gram-negative bacillary organisms (1, 4, 7). Although preliminary in vitro data suggested that TMP plus sulfamethoxazole (SMZ) was also effective against *N. gonorrhoeae* (4, 7, 8, 13), a wide variety of media and conditions were used to obtain this information. The fastidious nature of the gonococcal organism, and the potential interaction of various media constituents with TMP-SMZ, make it imperative that a standardized method be utilized to meaningfully evaluate the efficacy of this agent against this bacterium. To investigate this problem, the in vitro susceptibility of 100 clinical isolates of *N. gonorrhoeae* to TMP-SMZ, in a combination of one part TMP and 19 parts SMZ, was determined on three different media under similar conditions, using an agar dilution technique.

MATERIALS AND METHODS

Isolation of N. gonorrhoeae. All gonococci tested were clinical isolates obtained from the endocervix of female patients with uncomplicated gonorrhea examined at Harbor General Hospital and neighboring clinics. Organisms were cultured on Thayer-Martin medium (21) at 35 to 37 C for 24 to 48 h in candle jars. Identification of the isolates was by typical appearance on Gram stain, oxidase reaction, and sugar fermentation reactions. Organisms were then stored at −70 C in a mixture of 50% horse serum and 50% Trypticase soy broth until ready for susceptibility testing (22).

Preparation of media antibiotics. Mueller-Hinton (BBL), Oxoid diagnostic sensitivity test (DST), and GC agars (BBL, GC agar base) were freshly prepared and enriched with 1% IsoVitaleX (BBL). Five-percent lysed horse blood (Scott Lab., Fiskeville, R.I.) was incorporated into each medium. The horse blood was lysed by the addition of 1:50 of a 10% saponin solution (7). Serial twofold dilutions of TMP and SMZ (provided by Hoffman LaRoche, Inc.) in a 1:19 combination starting with 5 μg of TMP per ml and 95 μg of SMZ per ml were prepared and incorporated into each medium.

Susceptibility testing. Susceptibility of *N. gonorrhoeae* to TMP-SMZ was determined by an agar dilution technique previously described (22). The organisms were thawed and subcultured on enriched chocolate agar for 24 h. Gonococcal suspensions were prepared in Mueller-Hinton broth from surface colonies on chocolate agar and adjusted to a McFarland no. 1 nephelometer standard (5), previously determined to approximate 2×10^6 colony-forming units per ml. Inocula of approximately 0.0026 ml (5.2×10^3 colony-forming units) were delivered using a Steers replicating apparatus (20). All plates were incubated at 35 to 37 C in candle jars and flooded with oxidase (Difco) at 24 h. The mini-

mal inhibitory concentration (MIC) recorded was the last TMP–SMZ concentration that completely inhibited visible growth of any discrete oxidase-positive colonies. Separate strains of *Staphylococcus aureus* ATCC-25923, *Escherichia coli* ATCC-25922, and *Streptococcus faecalis* CN 478 (kindly donated by S. R. M. Busby) with known MICs to TMP–SMZ were included in each determination for reproducibility.

RESULTS

The MICs of TMP–SMZ in Oxoid DST, Mueller-Hinton, and GC agars against 100 gonococcal isolates, and the cumulative percentage of organisms inhibited at each concentration, are summarized in Table 1. On Oxoid DST medium, 95% of the organisms were susceptible to TMP–SMZ at 2.5 to 47.5 μg/ml or less, whereas a slightly smaller percentage was susceptible in Mueller-Hinton (90%) and GC agars (84%). With the exception of three isolates on GC agar, none were resistant to TMP–SMZ at concentrations greater than 5 to 95 μg/ml. However, it should be added that the three nongonococcal control organisms failed to have a distinct end point on GC agar, with one or two colonies being present at TMP–SMZ concentrations of 5 to 95 μg/ml.

DISCUSSION

In an attempt to better correlate and evaluate information regarding susceptibility of *N. gonorrhoeae* to TMP–SMZ, in vitro susceptibility studies were performed using three different media concurrently under similar conditions. Oxoid DST agar was selected because of its low thymidine content (7); Mueller-Hinton and GC agars were chosen because of their frequent use for susceptibility studies of other antibiotics against *N. gonorrhoeae* (2, 14, 22). All media were enriched with 1% IsoVitaleX and prepared with 5% lysed horse blood to inactivate thymidine (to thymine) which may interfere with action of TMP and SMZ (7). TMP and SMZ were incorporated into the media at a 1:19 ratio. Based on in vivo pharmacokinetic studies (11), this approximates the blood-level ratio of the two compounds when ingested in the commercially available form of one part TMP to five parts SMZ. Inoculum size was kept uniform utilizing a Steers replicating apparatus, and all plates were read at 24 h.

With utilization of a uniform methodology, the in vitro susceptibility of 100 gonococcal isolates to TMP–SMZ was relatively similar on all three media. The percentage of organisms with MICs to TMP (as TMP–SMZ) in the range of 0.16 to 2.5 μg/ml to Oxoid DST, Mueller-Hinton, and GC media were 95, 90, and 84%, respectively. This is not dissimilar from previously reported susceptibility data for *N. gonorrhoeae* against TMP (as TMP–SMZ) (4, 7). These MIC values are well within the range of achievable blood levels of TMP previously reported after ingestion of TMP (as TMP–SMZ in 1:5 ratio) in single doses of 240 and 400 mg (6, 11). Additionally, the peak blood levels of SMZ in these studies were between 50 and 60 μg/ml at the time serum concentrations of TMP were 2.5 to 3.0 μg/ml. Therefore, the concentrations of both TMP and SMZ required for inhibition of most strains of *N. gonorrhoeae* can be easily achievable in the blood.

It would appear then that, with a uniformly standardized methodology, the in vitro susceptibility data of this organism to TMP–SMZ are comparable despite differences in types of media used. However, with GC agar the control organisms, *S. aureus*, *E. coli*, and *S. faecalis*, were not completely inhibited at concentrations up to 5 to 95 μg/ml of TMP–SMZ; usually one to two colonies were still visible. Furthermore, the slightly greater number of gonococcal isolates on GC agar (as compared to DST or Mueller-Hinton) with TMP–SMZ concentrations of 5 to 95 μg/ml or higher may suggest that this medium may be less desirable than the other two.

TABLE 1. *Susceptibility of 100 isolates of N. gonorrhoeae to TMP-SMZ (1:19 combination) by agar dilution*

Medium	Concentration of TMP-SMZ (μg/ml)										
	0.01–0.19	0.02–0.38	0.04–0.75	0.08–1.49	0.16–2.97	0.32–5.94	0.63–11.88	1.25–23.75	2.5–47.5	5–95	>5–>95
Oxoid DST				1[a]		10	35	30	19	5	
				(1)[b]		(11)	(46)	(76)	(95)	(100)	
Mueller-Hinton						4	19	39	28	10	
						(4)	(23)	(62)	(90)	(100)	
GC agar					4	7	19	34	20	13	3
					(4)	(11)	(30)	(64)	(84)	(97)	(100)

[a] Number of strains inhibited.

[b] Numbers in parentheses indicate cumulative percentage.

Preliminary clinical trials with TMP–SMZ in treating uncomplicated gonorrhea appear promising (3, 13, 18, 23). Moreover, with favorable in vitro susceptibility data which can be meaningfully interpreted, TMP–SMZ may become a useful addition to presently available armamentarium for treating uncomplicated gonococcal infections.

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