ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1990, p. 1303–1307
0066-4804/90/071303-05$02.00/0

Vol. 34, No. 7

MINIREVIEWS

Antimicrobial Susceptibility of *Haemophilus ducreyi*

YUSUF DANGOR,* RONALD C. BALLARD, STEVEN D. MILLER, AND HENDRIK J. KOORNHOF

Emergent Pathogen Research Unit of the South African Medical Research Council, School of Pathology, University of the Witwatersrand, and South African Institute for Medical Research, P.O. Box 1038, Johannesburg 2000, South Africa

INTRODUCTION

Chancroid, a sexually transmitted disease caused by *Haemophilus ducreyi*, is common in many tropical and subtropical countries of Africa (15, 32, 39, 45) and Southeast Asia (51, 61, 63) and has been associated with isolated outbreaks of genital ulcer disease in both North America (57) and Europe (43). It is the most common cause of sexually acquired genital ulceration in Africa, accounting for 80% of cases seen in Nairobi, Kenya (44), 52% of cases in The Gambia (35), more than 50% of cases in Johannesburg (15) and Durban (12), South Africa, and 39% of cases in Harare, Zimbabwe (32). The disease characteristically presents as painful ulcers of the genitalia which may often be found in association with painful regional lymphadenopathy.

The significance of the disease was recently enhanced with the finding that chancroid may be an important cofactor in the heterosexual transmission of human immunodeficiency virus infection (30). Recent studies undertaken in Nairobi indicate that 15% of patients with chancroid have also been infected with this virus (40) and that human immunodeficiency virus can be isolated from the exudate of chancroid lesions (31).

In recent years, stimulated by the work of Hammond et al. (23), selective culture techniques for the isolation of *H. ducreyi* have been developed (15, 45). In Kenya, the sensitivity of isolation of *H. ducreyi* from presumptive chancroid lesions has increased to over 80% when two such selective media are used (46). The introduction of these improved isolation techniques has also made it possible to correlate clinical and bacteriologic responses following therapy and to perform in vitro antimicrobial susceptibility tests (6, 17, 62).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

(i) Methodology. The standards of the National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing of fastidious organisms, including members of the genus *Haemophilus* (42), cannot be applied to *H. ducreyi* since this organism requires hemin and supplementation with other nutrients for growth. In addition, the cohesiveness of *H. ducreyi* colonies renders standardization of inoculum size difficult and the slow and differing growth rates of isolates results in failure to predictably reproduce the logarithmic growth phase.

With one exception, in which the authors used a microdilution technique (29), all MIC determinations recorded to date have employed the agar dilution method (6, 8, 18, 19, 23, 26, 48, 56, 58, 60, 62). Homogenization of the inoculum has generally been achieved by scraping colonies from the surface of the agar plates and suspending the organisms in Mueller-Hinton broth (8, 13, 16, 17, 23, 41, 48, 63). Either 10% fetal calf serum alone (66) or 20% fetal calf serum together with 40% minimal essential medium has also been added to the Mueller-Hinton broth (56). In Belgium and Thailand, *H. ducreyi* has been grown in hemin-containing Mueller-Hinton broth supplemented with glucose, glutamine, cysteine, and bovine albumin fraction V (58) or horse serum and IsoVitaleX (62). The bacterial suspensions were vortex mixed for 3 to 30 s (6, 21, 23, 41, 48, 58, 62) or sonicated at 6 μm for 10 to 15 s (13, 22, 26) and were allowed to stand for 15 min, after which they were compared with a 0.5 McFarland opacity standard (13, 19, 23, 41, 50, 56, 60). The inoculum size ranged from 10^4 CFU (58) to 10^5 CFU (6, 13, 21, 23, 41, 50, 56, 62), 10^6 CFU (7, 18, 22, 63), and 10^7 CFU (66).

Most centers have used Mueller-Hinton agar (6, 7, 56, 62) or gonococcal agar-based medium (13, 23, 41, 50, 49, 58, 60) for MIC determinations. Initially, gonococcal agar base was preferred when sulfonamides were being tested (6, 8, 23, 50), but more recently Mueller-Hinton agar base has been used (18, 62). Both media are enriched with 0.1% glucose, 0.01% glutamine, 0.025% hemin, and 5% lysed horse blood. The incubation temperatures used by various centers have ranged from 33°C (7, 8, 19, 23, 26, 66) to 35°C (13, 18, 20, 58, 62) and 37°C (55), the percent atmospheric CO_2 has ranged from 5% (22, 58, 62) to 10% (17, 19, 23, 41), and the duration of incubation has ranged from 24 h (6, 55) to 48 h (17, 19, 23, 60, 62) and 96 h (22, 26).

With the exception of sulfonamides and trimethoprim, consensus has been reached that endpoint determinations should be based on the lowest concentration of antibiotic which yields no growth, two single colonies, or a fine, barely visible haze on the agar surface (13, 19, 23, 56, 60). The endpoint determinations of sulfonamide and trimethoprim susceptibility tests have usually been based on a significant decrease in growth (±80%) compared with that seen on a control plate (13, 17, 50, 63).

For reasons of cost and convenience, MIC testing is usually performed in batches. Isolates may be frozen at −70°C in defibrinated rabbit blood or lyophilized in brain heart infusion broth containing defibrinated rabbit blood or in skim milk (20). The addition of 10% glycerol to skim milk has also been found to be suitable for storage of *H. ducreyi* at low temperatures (23). Only three papers describing disk diffusion techniques for susceptibility testing of *H. ducreyi* have appeared in the literature (10, 11, 19), and only Feltham and co-workers (19) have compared disk diffusion results with MIC determinations. Very large zones of inhibition (40 to 60 mm) have been obtained by using disks containing 15 μg of erythromycin, 30 μg of chloramphenicol, and 30 μg of cephalothin, while disks containing 30 μg of tetracycline give

* Corresponding author.

TABLE 1. Susceptibilities of *H. ducreyi* to various antimicrobial agents

Antimicrobial agent	MIC_{50} (μg/ml) (range)				
	Kenya (8, 18, 22, 34, 53)[a]	South Africa (1, 13[b])	France (55, 56)	The Netherlands (60)	Thailand (62)
Penicillin G	128 (4.0–>128)	16 (0.5–≥128)		≤8 (0.018–≥8.0)[c]	
Amoxicillin		64 (1.0–≥128)	64 (0.25–≥128)	≤16 (0.03–>16.0)	
Amoxicillin-clavulanic acid		1.0 (≤0.06–8.0)	1.0 (0.06–2.0)		
Cefoxitin	2.0 (1.0–4.0)	2.0 (≤0.5–16.0)	1.0 (0.5–2.0)		
Cefotaxime	0.0004 (≤0.002–0.016)	0.008 (≤0.004–0.06)	0.004 (0.004–0.016)	<0.03 (≤0.03–2)	
Ceftriaxone	0.002 (0.001–0.004)	≤0.002 (≤0.002–0.008)			0.015 (0.0007–0.007)
Ceftetrame	0.015 (0.001–0.03)	0.015 (≤0.008–0.125)			
Cefaclor	16.0 (4.0–32.0)				
Cefamandole	0.5 (0.031–4.0)		1.0 (0.06–8.0)		
Ceftazidime	0.063 (0.016–0.125)	0.03 (≥0.008–0.5)			
Cefoperazone	0.063 (0.016–0.05)				
Tetracycline	16.0 (8.0–64)	16.0 (0.5–≥128)	32 (8.0–64)	16 (<0.125–32)	>32 (>32)
Doxycycline	8.0 (1.0–32)	2.0 (≤0.25–64)	8.0 (0.25–32)		
Minocycline		0.25 (0.015–8.0)	1.0 (1.0–2.0)		
Spectinomycin	8.0 (8.0–32)	2.0 (0.015–64)	4.0 (2.0–4.0)		8.0 (4.0–16)
Kanamycin		2.0 (≤1.0–16)	2.0 (2.0–1024)	0.25 (<0.125–2.0)	>250 (>250)
Gentamicin			1.0 (0.5–1.0)	<0.125 (<0.125–0.5)	
Streptomycin			2.0 (2.0–256)		
Erythromycin	0.06 (0.004–0.12)	0.004 (0.002–0.125)	0.016 (0.002–0.03)	≤0.003 (<0.003–0.015)	0.015 (0.007–0.06)
Clarithromycin		0.004 (≤0.002–0.06)			
Roxithromycin			0.06 (0.016–0.06)		
Rifampin	0.008 (0.002–1.0)	0.004 (≤0.001–0.125)	0.008 (0.004–0.03)		
Chloramphenicol		0.5 (0.25–32)	4.0 (0.12–16)	0.25 (≤0.125–16)	8.0 (4.0–16)
Thiamphenicol		0.25 (≤0.125–32)			
Ciprofloxacin	0.004 (0.0005–0.03)	0.008 (≤0.004–0.125)	0.016 (0.004–0.016)	<0.003 (<0.003–0.03)	0.001 (<0.0005–0.001)
Rosoxacin	≤0.002 (0.001–0.004)	0.015 (≤0.004–0.125)	0.03 (0.016–0.06)		0.03 (0.3–0.06)
Pefloxacin				0.03 (<0.003–1.0)	
Ofloxacin			0.03 (0.03–0.06)		
Norfloxacin			0.06 (0.03–0.12)	0.03 (<0.003–0.5)	
Fleroxacin	0.03 (0.015–0.06)	0.06 (0.03–0.125)			
Trimethoprim	0.5 (0.125–2.0)	0.25 (≤0.125–16)		<0.125 (<0.125–8.0)	0.5 (0.12–>16.0)
Sulfamethoxazole	64 (8.0–1,024)	64 (4.0–≥128)		64 (<0.125–≥128)	>160 (>160)
TMP-SMX[d] (1:19)		0.25 (≤0.25–8.0)			

[a] Reference(s).
[b] Also Dangor et al., 15th ICC.
[c] Units per milliliter.
[d] TMP-SMX, Trimethoprim-sulfamethoxazole.

TABLE 2. Plasmids of *H. ducreyi*

Plasmid type	Resistance phenotype(s)[a]	Mass (MDa)	Geographical occurrence	Reference(s)
Antimicrobial resistance	Amp	7.0	Winnipeg, Kenya, Philippines	36, 37, 65
	Amp	5.7	Kenya, Mexico, South Africa, Winnipeg	2, 24, 64, 65
	Amp	3.2	Orange County, Calif.; Brazil	4, 65
	Su	4.9	Kenya	2
	Tc	30	Winnipeg, Kenya	4, 9
	Tc Cm	34	United States, Philippines, Singapore	52, 65
	Sm Km	3.1	France	53
Conjugative	None	23.5	Kenya	14

[a] Amp, Ampicillin resistance; Su, sulfonamide resistance; Tc, tetracycline resistance; Cm, chloramphenicol resistance; Sm, streptomycin resistance; Km, kanamycin resistance.

zone diameters of 18 to 26 mm (10, 19). It is clear that considerably more work should be done before disk diffusion techniques can be recommended for general use.

The chromogenic cephalosporin test for β-lactamase production (47) has generally been used by most centers.

(ii) Geographic distribution of antimicrobial susceptibilities. A comprehensive list of in vitro antimicrobial susceptibilities obtained worldwide is given in Table 1. Rates of production of β-lactamase by *H. ducreyi* range from 47% in The Netherlands (60) to virtually 100% in other countries (13, 45, 62). *H. ducreyi* strains have generally been found to be highly susceptible to most β-lactamase-stable cephalosporins, with MICs for 50% of strains tested (MIC_{50}s) as low as 0.002 μg/ml recorded for ceftriaxone. Cefotaxime is also very active against *H. ducreyi*, while the new oral cephalosporin ceftetrame has an MIC_{50} of 0.015 μg/ml, compared with 16 μg/ml for cefaclor. Most isolates of *H. ducreyi* worldwide are susceptible to erythromycin, but strains for which MICs are 4 μg/ml have been encountered in Singapore (59). *H. ducreyi* strains have also been found to be susceptible to the newer macrolides clarithromycin (MIC_{50}, 0.004 μg/ml) and roxithromycin (MIC_{50}, 0.06 μg/ml). The quinolones ciprofloxacin, ofloxacin, pefloxacin, rosoxacin, norfloxacin, and fleroxacin have all shown excellent in vitro activity against *H. ducreyi* (1, 2, 5, 16, 21, 34, 41, 60, 62, 66; Y. Dangor, L. D. Liebowitz, and H. J. Koornhof, Proc. 15th Int. Congr. Chemother., p. 1959–1960, 1987). Rifampin was shown to be highly active against *H. ducreyi* in three centers, with MIC_{50}s of 0.004 to 0.008 μg/ml. Two rifamycins, rifabutin and FCE 22250, with long half-lives are also active, with MICs for 90% of strains tested (MIC_{90}s) of 0.016 and 0.06 μg/ml, respectively (1). Strains of *H. ducreyi* from centers listed in Table 1, as well as from Sheffield, England, and Singapore (22, 59), have been found to be susceptible to spectinomycin. The spectinomycin analog trospectinomycin also exhibited excellent in vitro activity (MIC_{90}, 0.25 μg/ml) against 23 strains of *H. ducreyi* isolated in France (56). Canadian and African isolates of *H. ducreyi* were shown to be susceptible to kanamycin (58) but MICs of ≥16 μg/ml have recently been reported from South Africa, France, and Thailand (13, 56, 62). Although *H. ducreyi* isolates from Winnipeg, Manitoba, Canada, and from Belgium have been shown to be fully susceptible to chloramphenicol (23, 58) and thiamphenicol exhibited good activity against strains isolated in Zimbabwe (33), chloramphenicol-resistant strains producing chloramphenicol acetyltransferase (54) have been reported from South Africa; the Philippines; Paris, France; Amsterdam, The Netherlands; and Bangkok, Thailand (13, 24, 54, 60, 62).

Trimethoprim resistance has frequently been detected in the United States (57), and high-level trimethoprim resistance has also been documented in the Far East (63). African strains have been shown to be relatively susceptible to trimethoprim, with only one strain found to be resistant in Nairobi (MIC, 32 μg/ml) (50). However, 14% of recent isolates from South Africa exhibited in vitro resistance to trimethoprim (MIC, 4 μg/ml) (13). In contrast to the experience in Thailand (62), where MICs for most strains are >160 μg/ml, some *H. ducreyi* isolates in Kenya, South Africa, and The Netherlands are susceptible to sulfamethoxazole. The combination of trimethoprim-sulfamethoxazole was tested in South Africa and shown to be synergistic (28). However, for 13% of strains MICs were recently found to be >2/38 μg/ml (13).

Based on breakpoints of 4 μg/ml, tetracycline resistance is widespread in Thailand (MIC_{50}, >32 μg/ml), France (MIC_{50}, 32 μg/ml), The Netherlands (MIC_{50}, 16 μg/ml), and South Africa (MIC_{50}, 16 μg/ml), but resistance to doxycycline and especially minocycline is less common (13, 56). In two studies conducted in France (55, 56), all strains were found to be susceptible to minocycline; more recently, 10% of strains in South Africa were found to be resistant to this tetracycline analog (13). In these studies, minocycline had MICs four to eight times lower than those of doxycycline. Doxycycline resistance has frequently been documented in Kenya (17), France (55), and South Africa (13), with MIC_{90}s of 32 μg/ml.

Several plasmids encoding antimicrobial resistance have been characterized, and these were reviewed by McNicol and Ronald (38). Small plasmids (7.0 megadaltons [MDa]) coding for ampicillin resistance (Amp^r) mediated by β-lactamase have been found in Canada, Kenya, and the Philippines (Table 2). Different Amp^r plasmids (5.7 MDa) were recorded from Canada, South Africa, Kenya, and Mexico, while a 3.2-MDa Amp^r plasmid has been found in strains from Brazil and Orange County, Calif. In contrast to the plasmids reported elsewhere (27), 3.95-, 5.2-, and 6.4-MDa plasmids in ampicillin-resistant strains of *H. ducreyi* in South Africa were found by Thomson and Bilgeri (64). Small plasmids coding for sulfonamide resistance and streptomycin-kanamycin resistance have also been found in Kenya and France, respectively, while larger plasmids (30 to 34 MDa) coding for tetracycline resistance and tetracycline-chloramphenicol resistance have been found in the United States, the Philippines, and Singapore (3, 52, 65).

Recently, Johnson and co-workers (25) from the Centers for Disease Control described a tetracycline-resistant, penicillin-resistant isolate of *H. ducreyi* that harbored the streptococcal tetracycline resistance determinant Tet M. The *tetM* gene was not plasmid borne but was located in the

bacterial chromosome. Since non-plasmid-mediated resistance to tetracycline has been documented in the absence of the *tetM* gene, it appears that tetracycline resistance in *H. ducreyi* may be acquired as a result of several distinct mechanisms. This could in part explain the differences in susceptibility of these organisms detected when the parent compound is compared with its analogs.

CONCLUSIONS AND RECOMMENDATIONS

Standardization of the methodology used for MIC determinations for *H. ducreyi* isolates is a high priority. In the meantime, the following procedures are proposed: (i) homogenizing colonies of *H. ducreyi* in Mueller-Hinton broth, (ii) mixing the bacterial suspension in a vortex mixer or by sonication and allowing the suspension to stand for 15 min, (iii) determining the concentration of the inoculum by comparison with a 0.5 McFarland standard opacity tube and subsequent adjustment to 10^5 CFU, (iv) using enriched Mueller-Hinton agar at an incubation temperature of 35°C in a humid atmosphere containing 5% CO_2, and (v) reading plates after incubation for 48 h and endpoints based on the lowest concentration of the antibiotic which yields no growth, two single colonies, or a fine, barely visible haze (or, for sulfonamide and trimethoprim testing, a ±80% decrease in growth compared with that on control plates).

Although very little experience with disk diffusion techniques has been documented, disk diffusion could be developed into a useful screening method for antimicrobial susceptibilities. In this respect, attention will have to be paid to the evaluation of disks with lower-than-usual concentrations of antimicrobial agents and the preparation of the inoculum.

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