ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1992, p. 1639–1643
0066-4804/92/081639-05$02.00/0

Vol. 36, No. 8

Correlation between In Vitro Antimicrobial Susceptibilities and β-Lactamase Plasmid Contents of Isolates of *Haemophilus ducreyi* from the United States

MILWOOD MOTLEY,[1]* SAMUEL K. SARAFIAN,[2] JOAN S. KNAPP,[2] AKBAR A. ZAIDI,[3] AND GEORGE SCHMID[3]

Department of Microbiology and Immunology, Morehouse School of Medicine, Atlanta, Georgia 30310,[1] and Division of Sexually Transmitted Diseases Laboratory Research, National Center for Infectious Diseases,[2] and Division of STD/HIV Prevention, National Center for Prevention Services,[3] Centers for Disease Control, Atlanta, Georgia 30333

Received 12 February 1992/Accepted 5 June 1992

We determined the susceptibilities of 94 strains of *Haemophilus ducreyi* isolated in various municipalities in the United States between 1982 and 1989 to the following antimicrobial agents: amoxicillin-clavulanic acid, ceftriaxone, erythromycin, azithromycin, ciprofloxacin, ofloxacin, trimethoprim, and spectinomycin. Ceftriaxone (MIC, ≤0.008 μg/ml), azithromycin (MIC, ≤0.125 μg/ml), erythromycin (MIC, ≤0.125 μg/ml), ciprofloxacin (MIC, ≤0.25 μg/ml), and ofloxacin (MIC, ≤0.25 μg/ml) were highly active against all isolates. Amoxicillin-clavulanic acid (MICs, 0.25 to 8.0 μg/ml), trimethoprim (MICs, 0.06 to 16.0 μg/ml), and spectinomycin (MICs, 2.0 to ≥32.0 μg/ml) were less active against these isolates. Isolates possessing the 5.7-MDa β-lactamase plasmid were less susceptible to erythromycin, trimethoprim, and spectinomycin than were isolates possessing the 3.2-MDa β-lactamase plasmid. The susceptibilities of plasmidless isolates to erythromycin, trimethoprim, and spectinomycin were distributed bimodally; the median MIC for the more susceptible plasmidless isolates corresponded to that for isolates with the 3.2-MDa plasmid, and the median MIC for the less susceptible plasmidless isolates corresponded to that for isolates with the 5.7-MDa plasmid. Thus, plasmid profiles may be valuable markers for geographical variations in antimicrobial susceptibilities of *H. ducreyi* strains that may indicate the relative efficacy of regimens for the treatment of chancroid. Of the regimens recommended by the U.S. Public Health Service for the treatment of chancroid, our results support the use of erythromycin, ceftriaxone, and ciprofloxacin, and perhaps ofloxacin, but suggest that amoxicillin-clavulanic acid and sulfamethoxazole-trimethoprim should be used with caution.

Chancroid, caused by *Haemophilus ducreyi*, is characterized by painful genital ulcers and tender inguinal lymph nodes. Although chancroid has been common in developing countries of the world, it is now diagnosed with increasing frequency in industrialized countries, including the United States and Canada (5, 14). In the United States, as a result of increasingly frequent outbreaks of chancroid that began in 1981, the reported number of cases in the United States has increased from 521 in 1978 to 4,215 in 1990 (6, 28).

The reported antimicrobial susceptibilities of isolates of *H. ducreyi* vary in different countries (19, 30). The differences in antimicrobial susceptibilities among isolates may be due either to intrinsic differences in the susceptibilities of isolates from different geographic areas or to differences in the procedures used to determine susceptibilities, as has been noted previously for *Neisseria gonorrhoeae* (8, 23). In the United States and Canada, antimicrobial susceptibilities have usually been determined for isolates of *H. ducreyi* from individual local outbreaks. Thus, it is possible that many isolates of the same strain have been tested in contrast to testing isolates of many different strains (15, 28).

To evaluate the in vitro susceptibilities of *H. ducreyi* to agents of current and potential use for the treatment of chancroid, we determined the susceptibilities of isolates of *H. ducreyi* collected between 1982 and 1989 from 11 cities in the United States to eight antimicrobial agents, and we correlated antimicrobial susceptibilities with the plasmid contents of the isolates. We hypothesized that such a correlation, if it existed, would allow us to use plasmid content as a surveillance marker to predict the antimicrobial activity of therapeutic agents.

MATERIALS AND METHODS

Strains. A total of 94 isolates of *H. ducreyi* were tested; these isolates were collected between 1982 and 1989 in 11 cities in the United States (Table 1). In addition, the type strain of *H. ducreyi* (HD-175; CIP 542) was tested as a reference strain. The isolates were frozen at −70°C in brain heart infusion broth with 10% glycerol.

Antimicrobial susceptibilities. The susceptibilities of isolates to amoxicillin-clavulanic acid (2:1) (Beecham Laboratories, Briston, Tenn.), ceftriaxone (Hoffmann-LaRoche, Nutley, N.J.), erythromycin (Eli Lilly, Indianapolis, Ind.), azithromycin (Pfizer, Inc., Groton, Conn.), ciprofloxacin (Miles Laboratories, West Haven, Conn.), ofloxacin (Ortho Pharmaceutical Corp., Raritan, N.J.), trimethoprim (Hoffmann-LaRoche), and spectinomycin (Upjohn, Kalamazoo, Mich.) were determined by a modification of the procedure described by Hammond et al. (13). The isolates were grown on gonococcal medium base (Difco Laboratories, Detroit, Mich.) supplemented with 1% hemoglobin (Difco), 1% IsoVitaleX (Becton Dickinson), and 5% fetal bovine serum (Hazleton Laboratories, Lenexa, Kans.) at 33°C in a humid atmosphere supplemented with 5% CO_2. Growth from 48-h

* Corresponding author.

TABLE 1. Geographic sources and plasmid contents of 94 isolates of *H. ducreyi* for which antimicrobial susceptibilities were determined

Geographic origin	Year(s) of isolation	No. of isolates	No. of isolates with plasmid		
			None	3.2 MDa	5.7 MDa
Tampa, Fla.	1987–1989	51	20		31
Long Beach-Los Angeles, Calif.	1987	21	5	7	9
New Orleans, La.	1989	10	1		9
Nashville, Tenn.	1989	3			3
Orange County, Calif.	1982	2	1	1	
Atlanta, Ga.	1982, 1989	2			2
Houston, Tex.	1989	2			2
Jacksonville, Fla.	1989	1			1
San Francisco, Calif.	1989	1			1
West Palm Beach, Fla.	1984	1			1

cultures was suspended in 4 ml of brain heart infusion broth, vortexed, and allowed to sediment for at least 15 min. The supernatants were separated, and their optical densities were adjusted to that of a 0.5 McFarland barium sulfate standard. The adjusted suspensions were inoculated with a multipoint inoculator (Cathra Systems, MCT Medical, St. Paul, Minn.) to supplemented gonococcal base medium containing twofold serial dilutions of the antimicrobial agents; the plates were incubated at 33°C in a humid atmosphere containing 5% CO_2. After 48 h, the MICs of the agents for the isolates were recorded. The MIC of an antibiotic for each isolate was recorded as the lowest concentration that inhibited its growth; any isolate exhibiting three or fewer colonies or a faint haze caused by inoculum was considered inhibited. The significance of the MICs was determined by chi-square tests.

Plasmid profiles. The plasmid profiles of the isolates were determined as described previously (22).

RESULTS

The susceptibilities of 94 isolates of *H. ducreyi* to amoxicillin-clavulanic acid, ceftriaxone, erythromycin, azithromycin, ciprofloxacin, ofloxacin, trimethoprim, and spectinomycin were determined (Table 2). The MICs to which the reference strain, HD-175, was susceptible were the following: amoxicillin-clavulanic acid, 2.0 μg/ml; ceftriaxone, ≤0.008 μg/ml; erythromycin, ≤0.008 μg/ml; azithromycin, 0.031 μg/ml; ciprofloxacin, ≤0.008 μg/ml; ofloxacin, 0.031 μg/ml; trimethoprim, 8.0 μg/ml, and spectinomycin, 32.0 μg/ml. Ceftriaxone, erythromycin, azithromycin, ciprofloxacin, and ofloxacin exhibited high in vitro activity against all isolates tested. Ceftriaxone was the most active of the agents; all isolates were susceptible to ≤0.008 μg of ceftriaxone per ml. Azithromycin was more active than erythromycin ($P < 0.01$), and ciprofloxacin was more active than ofloxacin ($P < 0.01$).

When the isolates were considered as a group, their susceptibilities to erythromycin, trimethoprim, and spectinomycin appeared to be distributed bimodally (Table 2).

TABLE 2. Correlation between susceptibilities to seven antimicrobial agents and β-lactamase plasmid contents of 94 isolates of *H. ducreyi* from the United States

Agent and plasmid content[a]	No. of isolates for which MIC (μg/ml) was:													MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
	≤0.008	0.016	0.031	0.062	0.125	0.25	0.5	1.0	2.0	4.0	8.0	16.0	32.0		
A/C															
None						1		2	17	5	2			2.0	4.0
3.2 MDa										4	4			4.0	8.0
5.7 MDa								3	14	35	7			4.0	8.0
Ery															
None	17	4	1	4	1									≤0.008	0.062
3.2 MDa	8													≤0.008	≤0.008
5.7 MDa	9		6	40	4									≤0.062	0.062
Az															
None	20	5	1		1									≤0.008	0.016
3.2 MDa	8													≤0.008	≤0.008
5.7 MDa	13	44	2											0.016	0.016
Cip															
None	13	8	5			1								0.016	0.031
3.2 MDa	8													≤0.008	≤0.008
5.7 MDa	49	9	1											≤0.008	0.016
Ofl															
None	2	15	8	1		1								0.016	0.031
3.2 MDa		5	2	1										0.016	0.062
5.7 MDa	11	33	14	1										0.016	0.016
TMP															
None				2	5	7	2		2	3	6			0.25	8.0
3.2 MDa					1	3	3		2					0.5	2.0
5.7 MDa						2		7	20	12	14	4		4.0	8.0
Spc															
None									7	11	2	4	3	4.0	32.0
3.2 MDa										7	1			4.0	8.0
5.7 MDa										1	2	52	4	16.0	16.0

[a] All isolates were susceptible to ≤0.008 μg of ceftriaxone per ml. Abbreviations: A/C, amoxicillin-clavulanic acid; Ery, erythromycin; Az, azithromycin; Cip, ciprofloxacin; Ofl, ofloxacin; TMP, trimethoprim; Spc, spectinomycin.

When analyzed by plasmid content, isolates possessing the 5.7-MDa β-lactamase plasmid were found to be less susceptible to these agents than isolates possessing the 3.2-MDa β-lactamase plasmid or no plasmid ($P < 0.05$ for each agent) (Table 2). The susceptibilities of plasmidless isolates to trimethoprim clearly exhibited a bimodal distribution; the susceptibilities of these isolates to erythromycin and spectinomycin also appeared to be distributed bimodally. The median MICs for the more susceptible plasmidless isolates corresponded to those for isolates possessing the 3.2-MDa plasmid, whereas the median MICs for the less susceptible plasmidless isolates corresponded to those for isolates possessing the 5.7-MDa plasmid.

Because the association between possession of the 5.7-MDa β-lactamase plasmid and antimicrobial susceptibilities could have been due to chance geographical differences in the occurrence of "resistant" isolates that happened to have the 5.7-MDa plasmid, we examined the association between plasmid content and susceptibilities for isolates from two geographically diverse locations. In outbreaks in Long Beach and Los Angeles (1987) and Tampa (1988 to 1989), isolates possessing the 5.7-MDa plasmid exhibited similar susceptibilities to erythromycin, trimethoprim, and spectinomycin.

DISCUSSION

In this study, ceftriaxone, erythromycin, azithromycin, ciprofloxacin, and ofloxacin were highly active in vitro against all isolates tested. In contrast, amoxicillin-clavulanic acid, spectinomycin, and trimethoprim were less active in vitro against all isolates. The susceptibilities of the U.S. isolates fell within the range of those in previous reports (1, 7, 10, 12, 16, 24, 27, 30, 31, 33). We observed, however, an association between plasmid content and antimicrobial susceptibilities. Isolates with the 5.7-MDa β-lactamase plasmid were less susceptible to erythromycin, spectinomycin, and trimethoprim than were isolates that possessed the 3.2-MDa β-lactamase plasmid. The median MICs indicated by the peaks identified in the bimodal distribution of susceptibilities of isolates with no plasmids corresponded to the median MICs for the isolates with 5.7- and 3.2-MDa β-lactamase plasmids, respectively.

During the past decade, plasmid-mediated resistance to tetracyclines, sulfonamides, and penicillins has been described worldwide among *H. ducreyi* strains. Three β-lactamase plasmids, with molecular sizes of 3.2, 5.7, and 7.0 MDa, have been described in *H. ducreyi*. The 7.0- and 5.7-MDa plasmids differ only in the presence of a 1.3-MDa insertion sequence in the former (4, 21). The 3.2-MDa plasmid of *H. ducreyi* is identical to the gonococcal 3.2-MDa β-lactamase plasmid (2) and is distinguished from the 5.7-MDa plasmid by lacking part of the TnA sequence (32). The 5.7-MDa β-lactamase plasmid in *H. ducreyi* differs from the gonococcal 4.7-MDa β-lactamase plasmid by possessing the entire TnA sequence.

There are no reports showing that the β-lactamase plasmids of *H. ducreyi* or *N. gonorrhoeae* confer resistance to antimicrobial agents other than β-lactam antibiotics. It is fascinating, however, to observe an association between the presence of the 5.7-MDa β-lactamase plasmid and decreased susceptibility to unrelated antimicrobial agents similar to the correlation between the presence of the 4.4-MDa β-lactamase plasmid and chromosomal antimicrobial resistance observed in *N. gonorrhoeae* (34). Isolates of *N. gonorrhoeae* that possess the 4.4-MDa β-lactamase plasmid, usually considered to have originated in the Far East, are frequently resistant to tetracycline, erythromycin, and expanded-spectrum cephalosporins and exhibit decreased susceptibility to broad-spectrum cephalosporins such as ceftriaxone. In contrast, penicillin-producing *N. gonorrhoeae* strains that possess the 3.2-MDa β-lactamase plasmid, which originated in Africa, are susceptible to these agents (34). The resistances of the gonococcus to non-β-lactam antimicrobial agents are due to chromosomal mutations (9) and are, thus, intrinsic characteristics of gonococcal isolates. For example, gonococcal isolates from the Far East are more resistant to antimicrobial agents than those from other geographic areas (18). There may be similar correlations between antimicrobial resistance and geographic origin of *H. ducreyi* strains.

Geographic variations among MICs of agents for *H. ducreyi* isolates have been described: for example, the susceptibilities of *H. ducreyi* isolates to trimethoprim (1, 7, 9, 11, 23, 27, 29, 31). Such differences might be due to the various distribution of isolates with different plasmid contents. Although the isolates tested in our study required a wide range of MICs to trimethoprim, those possessing the 5.7-MDa β-lactamase plasmid required a higher MIC (MIC for 50% of isolates [MIC_{50}] = 4.0 μg of trimethoprim per ml) than isolates with either the 3.2-MDa plasmid or no plasmids (MIC_{50} = 0.25 and 0.5 μg/ml, respectively). Decreased susceptibilities to trimethoprim have been reported for β-lactamase-producing strains in South Africa and the Netherlands (1, 29). MICs of ≥2.0 μg trimethoprim per ml were recently reported in 92% of 38 South African isolates possessing either the 5.7- or the 7.0-MDa β-lactamase plasmid (1). In the United States, isolates with the 5.7-MDa plasmid were first reported in 1981 (15), and isolates with a pattern of decreased susceptibility to trimethoprim have been observed since 1982 (26). This recent increase in MICs of trimethoprim may reflect the predominance of isolates with the 5.7-MDa plasmid in the United States during the 1980s (25).

Similarly, variations in susceptibilities of isolates to spectinomycin have also been observed in different geographic areas (1, 7, 30). Since isolates with the 5.7-MDa β-lactamase plasmid were less susceptible to spectinomycin than those with the 3.2-MDa plasmid or those with no plasmids, it is possible that the geographic variation in spectinomycin susceptibilities might be accounted for by the prevalence of isolates with the 5.7-MDa plasmid.

Although the susceptibilities of *H. ducreyi* isolates in our study were usually similar to those previously reported, some variations in the range of susceptibilities were noted. These variations could be explained by either the procedures used to measure susceptibilities or the distribution of isolates with truly different antimicrobial susceptibilities. Since we have demonstrated differences in susceptibility patterns that correlate with plasmid content, such as those described above, it is reasonable to speculate that the distribution of isolates with different plasmid profiles may account for some of the variations noted in previous studies. Further studies that compare the procedures for determining susceptibilities and that correlate susceptibility patterns with plasmid profiles will be required to elucidate definitively the basis for geographic differences in antimicrobial susceptibility patterns among *H. ducreyi* isolates.

In the United States, several regimens are currently recommended for treating chancroid. The primary recommendations are either erythromycin base (500 mg orally four times a day for 7 days) or ceftriaxone (250 mg intramuscularly in a single dose). Alternative regimens are trimethoprim-sulfamethoxazole (160/180 mg [one double-strength

tablet] orally two times a day for 7 days), amoxicillin (500 mg) plus clavulanic acid (125 mg, orally three times a day for 7 days), and ciprofloxacin (500 mg, orally two times a day for 3 days).

Our results indicate that erythromycin and ceftriaxone are very active in vitro against all isolates tested and support the use of these agents for the treatment of chancroid in the United States. Among the alternative regimens for which we determined in vitro susceptibilities, ciprofloxacin (and perhaps ofloxacin) would appear to be the most effective regimens because all isolates were highly susceptible to these agents. Although no criteria for the interpretation of antimicrobial susceptibilities of *H. ducreyi* have been established, these isolates would be considered resistant to amoxicillin-clavulanic acid, if resistance is interpreted by the same criteria used to interpret penicillin G susceptibility results for isolates of *N. gonorrhoeae* (resistance = MIC of ≥2.0 μg/ml) (17); thus, we recommend caution in the use of amoxicillin-clavulanic acid for the treatment of chancroid in the United States until in vivo trials are performed to evaluate the clinical efficacy of this regimen.

In contrast to amoxicillin-clavulanic acid, both in vitro and in vivo susceptibility data are available for ciprofloxacin and other quinolones (27). The greater in vitro activity of ciprofloxacin suggests it may be more clinically effective than other quinolones (28). Indeed, single-dose therapy with ciprofloxacin has been quite successful in Kenya and Thailand, whereas clinical trials in Kenya with fleroxacin, enoxacin, and rosoxacin suggest the need for multiple doses of these agents (20). Because human immunodeficiency virus infection, increasingly common in Kenya, apparently has an effect on treatment response among individuals infected with *H. ducreyi*, it is difficult to compare the results of treatment efficacy studies conducted at different times (28).

The susceptibilities of *H. ducreyi* isolates to sulfamethoxazole-trimethoprim (19:1) are similar to the susceptibilities to trimethoprim alone (3, 7). The range of susceptibilities of our isolates to trimethoprim suggest that caution should also be exercised in treating individuals with chancroid in the United States with sulfamethoxazole-trimethoprim, particularly in view of the decreased effectiveness of this regimen for treating chancroid in Thailand and perhaps Kenya (10, 31).

Azithromycin, a tissue-active macrolide of erythromycin, has previously been shown to be as active against *H. ducreyi* as erythromycin (16); our data show it to be more active. Although isolates possessing a 5.7-MDa β-lactamase plasmid were less susceptible to erythromycin and azithomycin (though remaining well within the therapeutically effective range), clinical studies comparing the efficacy of azithromycin with that of erythromycin are warranted, particularly to evaluate their efficacy against strains possessing the 5.7-MDa plasmid.

Monitoring the antimicrobial susceptibilities of *H. ducreyi* strains with various plasmid profiles and from various geographical areas is warranted. Plasmid profiles may be valuable markers for geographical variations in antimicrobial susceptibilities of *H. ducreyi* strains that may indicate the relative efficacy of regimens for the treatment of chancroid.

REFERENCES

1. **Abeck, D., A. Johnson, Y. Dangor, and R. Ballard.** 1988. Antibiotic susceptibilities and plasmid profiles of *Haemophilus ducreyi* isolates from southern Africa. J. Antimicrob. Chemother. **22**:437–444.
2. **Anderson, B., W. Albritton, J. Biddle, and S. Johnson.** 1984. Common β-lactamase-specifying plasmid in *Haemophilus ducreyi* and *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **25**:296–297.
3. **Bilgeri, Y. R., R. C. Ballard, M. O. Duncan, A. C. Mauff, and H. J. Kornhoff.** 1982. Antimicrobial susceptibility of 103 strains of *Haemophilus ducreyi* isolated in Johannesburg. Antimicrob. Agents Chemother. **22**:686–688.
4. **Brunton, J., M. Meier, N. Ehrman, I. Maclean, L. Slaney, and W. Albritton.** 1982. Molecular epidemiology of beta-lactamase-specifying plasmids of *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **21**:857–863.
5. **Centers for Disease Control.** 1988. Summary of notifiable diseases, United States, 1987. Morbid. Mortal. Weekly Rep. **36**:53.
6. **Centers for Disease Control.** 1990. Sexually transmitted diseases surveillance 1990. Centers for Disease Control, Atlanta, Ga.
7. **Dangor, Y., S. Miller, F. Exposta, and H. Koornhof.** 1988. Antimicrobial susceptibilities of South African isolates of *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **32**:1458–1460.
8. **Dillon, J. R., W. Tostowaryk, and M. Pauzé.** 1987. Effects of different media and methods of inoculum preparation on results of antimicrobial susceptibility testing of *Neisseria gonorrhoeae* by agar dilution. Antimicrob. Agents Chemother. **31**:1744–1749.
9. **Dillon, J. R., and K.-H. Yeung.** 1989. β-Lactamase plasmids and chromosomally mediated antimicrobial resistance in pathogenic *Neisseria* species. Clin. Microbiol. Rev. **2**(Suppl.):S125–S133.
10. **Dylewski, J., H. Nsanze, L. D'Costa, L. Slaney, and A. Ronald.** 1985. Trimethoprim sulphamoxole in the treatment of chancroid. Comparison of two single dose treatment regimens with a five day regimen. J. Antimicrob. Chemother. **16**:103–109.
11. **Fast, M., H. Nsanze, L. D'Costa, P. Karasira, I. Maclean, P. Piot, W. Albritton, and A. Ronald.** 1983. Antimicrobial therapy of chancroid: an evaluation of five treatment regimens correlated with in vitro sensitivity. Sex. Transm. Dis. **10**:1–6.
12. **Girouard, Y., I. Maclean, A. Ronald, and W. Albritton.** 1981. Synergistic antibacterial activity of clavulanic acid and amoxicillin against β-lactamase-producing strains of *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **20**:144–145.
13. **Hammond, G., C. Lian, J. Wilt, and A. Ronald.** 1978. Antimicrobial susceptibility of *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **13**:608–612.
14. **Hammond, G., M. Stutchuk, J. Scatcliff, E. Sherman, J. Wilt, and A. Ronald.** 1980. Epidemiologic, clinical, laboratory, and therapeutic features of an urban outbreak of chancroid in North America. Rev. Infect. Dis. **2**:867–879.
15. **Handsfield, H., P. Totten, C. Fennel, S. Falkow, and K. Holmes.** 1981. Molecular epidemiology of *Haemophilus ducreyi* infections. Ann. Intern. Med. **95**:315–318.
16. **Jones, B., G. Kinghorn, and B. Duerden.** 1988. In vitro activity of azithromycin and erythromycin against organisms associated with bacterial vaginosis and chancroid. Eur. J. Clin. Microbiol. Infect. Dis. **7**:551–553.
17. **Jones, R. N., T. L. Gavin, C. Thornsberry, P. C. Fuchs, E. H. Gerlach, J. S. Knapp, P. Murray, and J. A. Washington.** 1989. Standardization of disk diffusion and agar dilution susceptibility tests for *Neisseria gonorrhoeae*: interpretive criteria and quality control guidelines for ceftriaxone, penicillin, spectinomycin, and tetracycline. J. Clin Microbiol. **27**:2758–2766.
18. **Knapp, J. S., and K. K. Holmes.** 1975. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. J. Infect. Dis. **132**:204–208.
19. **Kraus, S., H. Kaufman, W. Albritton, C. Thornsberry, and J. Biddle.** 1982. Chancroid therapy: a review of cases confirmed by culture. Rev. Infect. Dis. **4**(Suppl.):S848–S856.
20. **Le Saux, N. M., L. A. Slaney, F. A. Plummer, A. R. Ronald, and R. C. Brunham.** 1987. In vitro activity of ceftriaxone, cefetamet (Ro 15-8074), ceftetrame (Ro 19-5247; T-2588), and fleroxacin (Ro 23-6240; AM-833) versus *Neisseria gonorrhoeae* and *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **31**:1153–1154.
21. **McNicol, P., and A. Ronald.** 1984. The plasmids of *Haemophilus ducreyi*. J. Antimicrob. Chemother. **14**:561–564.
22. **Meyers, J. A., D. Sanchez, S. Elwell, and S. Falkow.** 1976.

Simple agarose gel electrophoresis method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1529–1537.

23. **Morse, S.** 1989. Chancroid and *Haemophilus ducreyi*. Clin. Microbiol. Rev. **2**:137–157.
24. **Sanson-LePors, M., I. Casin, M. Ortenberg, and Y. Perol.** 1983. In vitro susceptibility of thirty strains of *Haemophilus ducreyi* to several antibiotics including six cephalosporins. J. Antimicrob. Chemother. **11**:271–280.
25. **Sarafian, S. K., and J. S. Knapp.** 1992. Molecular epidemiology, based on plasmid profiles, of *Haemophilus ducreyi* infections in the United States: results of surveillance, 1981–1990. Sex. Transm. Dis. **19**:35–38.
26. **Schmid, G.** 1986. The treatment of chancroid. JAMA **255**:1757–1762.
27. **Schmid, G.** 1990. The treatment of chancroid, 1989. Rev. Infect. Dis. **12**(Suppl.):S580–S588.
28. **Schmid, G., L. Sanders, J. Blount, and E. Alexander.** 1987. Chancroid in the United States. Reestablishment of an old disease. JAMA **258**:3265–3268.
29. **Sturm, A. W.** 1987. Comparison of antimicrobial susceptibility patterns of fifty-seven strains of *Haemophilus ducreyi* isolated in Amsterdam from 1978 to 1985. J. Antimicrob. Chemother. **19**:187–191.
30. **Taylor, D., P. Echeverria, S. Hanchalay, C. Pitarangsi, L. Slootmans, and P. Piot.** 1985. Antimicrobial susceptibility and characterization of outer membrane proteins of *Haemophilus ducreyi* isolated in Thailand. J. Clin. Microbiol. **21**:442–444.
31. **Taylor, D., C. Pitarangsi, P. Echeverria, K. Panikabutra, and C. Suvangse.** 1985. Comparative study of ceftriaxone and trimethoprim-sulfamethoxazole for the treatment of chancroid in Thailand. J. Infect. Dis. **152**:1002–1006.
32. **Totten, P., H. Handsfield, D. Peters, K. Holmes, and S. Falkow.** 1982. Characterization of ampicillin resistance plasmids from *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **22**:622–627.
33. **Wall, R., D. Mabey, C. Bello, and D. Felmingham.** 1985. The comparative in vitro activity of twelve 4-quinolone antimicrobials against *Haemophilus ducreyi*. J. Antimicrob. Chemother. **16**:165–168.
34. **Zenilman, J. M., W. L. Whittington, D. Frazier, R. J. Rice, and J. S. Knapp.** 1988. Penicillinase-producing *Neisseria gonorrhoeae* (PPNG) in Dade County, Florida: phenotypic characterization of isolates from 1983, 1984, and 1986. Sex. Transm. Dis. **15**:158–163.