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# In Vitro Susceptibilities of Isolates of *Haemophilus ducreyi* from Thailand and the United States to Currently Recommended and Newer Agents for Treatment of Chancroid

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**We determined the in vitro susceptibilities of 54 isolates of *Haemophilus ducreyi* from Thailand (29 isolates) and San Francisco (25 isolates) to penicillin G, tetracycline, amoxicillin-clavulanic acid, ceftriaxone, cefixime, erythromycin, azithromycin, ciprofloxacin, ofloxacin, and trimethoprim-sulfamethoxazole. Isolates were susceptible to ≤0.25 μg of ceftriaxone per ml, ≤0.5 μg of cefixime per ml, ≤0.125 μg of ciprofloxacin per ml, and ≤0.06 μg of ofloxacin per ml. Erythromycin was active against all isolates (MIC for 90% of isolates tested, 0.25 μg/ml), as was azithromycin (MIC, ≤0.06 μg/ml). In contrast, all but one isolate were resistant to ≥8.0 μg of tetracycline per ml, 11.1% of the isolates were resistant to ≥8.0 μg of amoxicillin-clavulanic acid per ml, and 40.9% of the isolates were resistant to trimethoprim-sulfamethoxazole (MIC, ≥4/76 μg/ml).**

*Haemophilus ducreyi* is the etiologic agent of chancroid, a genital ulcer disease that is characterized by painful genital ulcers and tender inguinal lymphadenopathy. Although chancroid was frequently reported in the United States in the preantibiotic era, relatively few cases were reported between 1948 and 1978 (4). Since the late 1970s, however, chancroid has been diagnosed with increased frequency; 4,212 cases were reported to the Centers for Disease Control and Prevention (CDC) in 1990 and 3,476 cases were reported in 1991 (4). Because *H. ducreyi* is difficult to isolate from clinical specimens, most cases of chancroid are diagnosed clinically (after diagnoses of syphilis and herpes simplex virus have been ruled out) by the appearance of the ulcers and the resolution of symptoms after treatment with one of several recommended antimicrobial agents (3, 15). The current primary therapeutic regimens recommended by the CDC for chancroid are erythromycin or ceftriaxone; alternative recommended therapies are trimethoprim-sulfamethoxazole (TMP-SMX), amoxicillin-clavulanic acid (A-C), or ciprofloxacin (3). Because most *H. ducreyi* infections are treated empirically, it is important to monitor the antimicrobial susceptibilities of representative isolates of *H. ducreyi* and assess the efficacies of recommended and investigational antimicrobial agents for chancroid.

Previously, we determined the susceptibilities of selected *H. ducreyi* strains isolated in the United States to selected antimicrobial agents and demonstrated that most antimicrobial agents were less active against strains that possessed a 5.7-MDa β-lactamase plasmid than against strains that possessed a 3.2-MDa β-lactamase plasmid (10). In the present study, we compared the in vitro activities of currently recommended and newer antimicrobial agents against *H. ducreyi* isolates from Thailand that possessed novel plasmids (13) and isolates from San Francisco, Calif.

We characterized the plasmid contents and antimicrobial susceptibility patterns of 54 isolates, 29 isolates from Thailand (isolated in 1984) and 25 isolates from San Francisco (isolated in 1989 and 1990) (Table 1). All isolates from Thailand and 17 isolates from San Francisco produced β-lactamase; 8 isolates from San Francisco were β-lactamase negative. The isolates were grown on gonococcal medium base (Difco Laboratories, Detroit, Mich.) supplemented with 1% hemoglobin (Difco), 1% IsoVitaleX (Becton Dickinson, Cockeysville, Md.), and 5% fetal bovine serum (Hazleton Laboratories, Lenexa, Kans.) at 33°C in a humid atmosphere supplemented with 5% $CO_2$. The isolates were frozen at −70°C in brain heart infusion broth with 10% glycerol.

The susceptibilities of isolates to the following antimicrobial agents were determined as described previously: penicillin G, cefixime, and tetracycline (Lederle Laboratories, Pearl River, N.Y.), A-C (2:1) (Beecham Laboratories, Briston, Tenn.), cefoxitin (Merck Sharp & Dohme, Rahway, N.J.), ceftriaxone (Hoffmann-LaRoche Inc., Nutley, N.J.), erythromycin (Eli Lilly & Co., Indianapolis, Ind.), azithromycin (Pfizer Inc., Groton, Conn.), ciprofloxacin (Miles Laboratories, West Haven, Conn.), and ofloxacin (Ortho Pharmaceutical Corp., Raritan, N.J.) (10). Antimicrobial agents were obtained as standard powders and were prepared according to the manufacturers' instructions. The susceptibilities of isolates to these agents were determined on gonococcal base medium (Difco) supplemented with 1% hemoglobin (Difco), 1% IsoVitaleX (Becton Dickinson), and 5% fetal bovine serum (Hazelton Laboratories) at 33°C in a humid atmosphere supplemented with 5% $CO_2$. Susceptibilities to TMP-SMX (Hoffmann-La Roche Inc.), which cannot be determined on a GC base medium containing *para*-aminobenzoic acid, were determined on Diagnostic Sensitivity Test agar (Oxoid, Columbia, Md.). Growth from 48-h cultures was suspended in 4 ml of brain heart infusion broth, vortexed, and allowed to sediment for 15 min. The optical density of each supernatant was adjusted to that of a 0.5 McFarland barium sulfate standard. The adjusted suspen-

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TABLE 1. Origin, plasmid content, and group assignment of *H. ducreyi* isolates

| Strain group | Plasmid content (MDa) | Geographic origin | No. of isolates |
|---|---|---|---|
| 1 | 1.8, 2.6, 2.8, 3.2 | San Francisco | 1 |
| | | Thailand | 12 |
| 1 | 1.8, 2.6, 2.8, 3.2, 5.7 | Thailand | 3 |
| 1 | 1.8, 2.8, 3.2 | Thailand | 3 |
| 2 | 2.8, 3.2, 4.4, 5.7 | Thailand | 2 |
| 2 | 2.8, 3.2, 5.7 | Thailand | 5 |
| 2 | 2.8, 3.2, 5.7, 7.4 | Thailand | 2 |
| 2 | 2.8, 3.2, 7.4 | Thailand | 2 |
| 2 | 3.2 | San Francisco | 11 |
| 2 | 4.4, 5.7 | San Francisco | 3 |
| 2 | 5.7 | San Francisco | 2 |
| 2 | None | San Francisco | 8 |

sions were inoculated onto media with a multipoint inoculator (Cathra Systems, AutoMed, Arden Hills, Minn.). The type strain of *H. ducreyi* (HD-175; CIP 542) (a plasmidless strain), strains HD-001 and HD-011 (3.2-MDa plasmid), and strain HD-187 (7.0-MDa plasmid) were tested as reference strains. Susceptibilities were interpreted according to the recommendations for *Haemophilus* spp. of the National Committee for Clinical Laboratory Standards (11).

The distribution of susceptibilities and the MICs of β-lactam agents for 50% ($MIC_{50}$) and 90% ($MIC_{90}$) of β-lactamase-positive and β-lactamase-negative isolates tested are given in Table 2. The β-lactams (penicillin G and A-C) and the broad-spectrum cephalosporins (ceftriaxone and cefixime) were less active against β-lactamase-positive isolates than against β-lactamase-negative isolates. Of these isolates, 19 of 54 (35.2%) were resistant to ≥4.0 μg of A-C per ml; 6 of 54 (11.1%) were resistant to ≥8.0 μg of A-C per ml. Ceftriaxone was the most active of the β-lactam agents against both β-lactamase-positive and -negative isolates ($MIC_{90}$, 0.004 μg/ml) compared with cefixime ($MIC_{90}$, 0.25 μg/ml). All but one isolate were resistant to ≥8.0 μg of tetracycline per ml, the remaining isolate was intermediate in resistance (MIC, 4.0 μg of tetracycline per ml).

Isolates were grouped as indicated in Table 1 for analyses of the activities of the macrolides, quinolones, and TMP-SMX. Group 1 isolates from Thailand and San Francisco, which possessed a 1.8-MDa plasmid, showed different susceptibility patterns to other antimicrobial agents than did other isolates from Thailand and most isolates from San Francisco (group 2).

No interpretive criteria are available for erythromycin or azithromycin MICs for *H. ducreyi* isolates. However, on the basis of the interpretive criteria for erythromycin MICs for other bacterial species (resistance, MIC of ≥8.0 μg/ml), all isolates were susceptible to this agent. Erythromycin was active against the majority of isolates ($MIC_{90}$, 0.25 μg/ml), although it was noticeably less active against group 1 isolates than against most group 2 isolates (Table 2). The activity of erythromycin against the two San Francisco isolates possessing the 5.7-MDa plasmid and one Thai isolate (plasmid content, 2.8, 3.2, 5.7, and 7.4 MDa) was similar to its activity against the group 1 isolates (MICs, 0.125 to 0.25 μg/ml). In vitro, azithromycin was more active than erythromycin against the tested isolates (Table 2).

In contrast, the quinolones exhibited an inverse range of activities against group 1 and 2 isolates compared with those exhibited by the macrolides. Both ciprofloxacin and ofloxacin were more active against group 1 isolates than against group 2 isolates (Table 2). Ciprofloxacin was more active ($MIC_{90}$, 0.008 μg/ml) than ofloxacin ($MIC_{90}$, 0.06 μg/ml). Only one Thai isolate was resistant to 2.0 μg of ciprofloxacin per ml.

TMP-SMX was active (MIC, ≥0.5/9.5 μg/ml) against 27 of 54 (50%) isolates, all of which belonged to group 2. Five (9.3%) isolates, including three group 1 and two group 2 isolates, were intermediate in susceptibility to TMP-SMX (MIC, 1/19 to 2/38 μg/ml). A total of 22 of 54 (40.9%) isolates, including 17 group 1 isolates and 5 group 2 isolates, were resistant to TMP-SMX (MIC ≥4/76 μg/ml).

In the present study, we found that the antimicrobial agents currently recommended by the CDC for primary treatment of chancroid—erythromycin and ceftriaxone—were active against all 54 isolates of *H. ducreyi* tested. However, 40.9% of isolates were resistant to TMP-SMX (MIC, ≥4/76 μg/ml) and 32.5% of isolates were resistant to amoxicillin-clavulanic acid (MIC, ≥4.0 μg/ml), which are agents that are still recommended by the CDC as alternative therapies for chancroid (3). Most TMP-SMX-resistant isolates were from Thailand; the isolate from San Francisco possessing the 1.8-MDa plasmid was also resistant to TMP-SMX. Most isolates were highly susceptible to ciprofloxacin, although one resistant isolate was identified. Newer antimicrobial agents—cefixime, azithromycin, and ofloxacin—were highly active against all isolates, although cefixime and ofloxacin were less active than ceftriaxone and ciprofloxacin.

The activities reported for A-C, ceftriaxone, erythromycin, azithromycin, ciprofloxacin, and ofloxacin against the isolates tested in the present study are similar to those reported previously for U.S. isolates with similar plasmid profiles (10) and to the susceptibilities of isolates reported elsewhere (1, 5–9, 12, 16). Agents were less active against isolates that possessed the 5.7-MDa plasmid than against isolates that possessed no plasmid or those that possessed the 3.2-MDa plasmid. In contrast, A-C and TMP-SMX were relatively inactive against several isolates from Thailand and one isolate from San Francisco that had plasmid profiles not previously identified among U.S. isolates (13, 14). The TMP-SMX-resistant isolates also exhibited decreased susceptibilities to quinolones. The clinical significance of these in vitro observations is not clear, but these data support the need to systematically monitor known and emerging drug resistance and potential therapeutic failures.

We found no evidence in the present study to suggest that TMP-SMX resistance in the tested isolates was associated with a 4.4-MDa plasmid, as was reported previously (2, 9). Our data showed that isolates that possessed the 4.4- and 5.7-MDa plasmids are highly susceptible to TMP-SMX (MICs, ≤0.015/0.3 μg/ml). All TMP-SMX-resistant isolates from Thailand possessed a 2.8-MDa plasmid; for isolates possessing a 1.8-MDa plasmid only, MICs were ≥2/38 μg of TMP-SMX per ml, and several Thai isolates possessing a 2.8-MDa plasmid were highly susceptible to TMP-SMX. The TMP-SMX-resistant isolates from San Francisco possessed either the 1.8-MDa plasmid or the 5.7-MDa plasmid. Thus, we speculate that the TMP-SMX resistance in these isolates may be chromosomally mediated, because no correlation could be made between the plasmid profile and the MIC that would indicate that the TMP-SMX resistance was plasmid mediated. If the TMP-SMX resistance in these isolates is plasmid mediated, multiple plasmids may be associated with TMP-SMX resistance in *H. ducreyi*, which is in contrast to the information presented in a previous report (2, 9).

TABLE 2. Correlation between susceptibilities to seven antimicrobial agents and β-lactamase plasmid contents of 54 isolates of *H. ducreyi* from the United States and Thailand

| Antimicrobial agent and plasmid content[a] | No. of isolates for which MIC (μg/ml) was: | | | | | | | | | | | | | | | | MIC (μg/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.002 | 0.004 | 0.008 | 0.015 | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 | 16.0 | 32.0 | 64.0 | 50% | 90% |
| PEN G | | | | | | | | | | | | | | | | | | |
| β-Lac+ | | | | | | | | | | | | 1 | | 1 | 3 | 39 | 64 | 64 |
| β-Lac− | | | | | | | | 1 | 5 | 2 | | | | | | | | |
| A-C | | | | | | | | | | | | | | | | | | |
| β-Lac+ | | | | | 1 | | | | | | 6 | 20 | 13 | 6 | | | 2.0 | 8.0 |
| β-Lac− | | | | | | | | | | | 6 | 2 | | | | | | |
| CRO | | | | | | | | | | | | | | | | | | |
| β-Lac+ | 15 | 27 | 2 | | | 1 | | 1 | | | | | | | | | 0.004 | 0.004 |
| β-Lac− | 8 | | | | | | | | | | | | | | | | | |
| CFX | | | | | | | | | | | | | | | | | | |
| β-Lac+ | | | | 1 | 17 | 22 | 1 | 3 | 2 | | | | | | | | 0.06 | 0.25 |
| β-Lac− | | | | | | 7 | 1 | | | | | | | | | | | |
| ERY | | | | | | | | | | | | | | | | | | |
| Group 1[b] | | | 1 | | | | 13 | 4 | | | | 1 | | | | | 0.125 | 0.25 |
| Group 2[c] | 18 | 3 | 9 | 2 | | | 2 | 1 | | | | | | | | | 0.002 | 0.015 |
| AZ | | | | | | | | | | | | | | | | | | |
| Group 1 | | | 3 | 5 | 11 | | | | | | | | | | | | 0.03 | 0.03 |
| Group 2 | 2 | 28 | 2 | | 2 | 1 | | | | | | | | | | | 0.004 | 0.008 |
| CIP | | | | | | | | | | | | | | | | | | |
| Group 1 | 16 | 2 | | | | | | | | | 1 | | | | | | 0.004 | 0.008 |
| Group 2 | | 33 | 1 | | | | 1 | | | | | | | | | | 0.008 | 0.008 |
| OFX | | | | | | | | | | | | | | | | | | |
| Group 1 | | 14 | 4 | | | | 1 | | | | | | | | | | 0.015 | 0.03 |
| Group 2 | | 1 | 29 | 3 | | 2 | | | | | | | | | | | 0.03 | 0.06 |
| TMP-SMX[d] | | | | | | | | | | | | | | | | | | |
| Group 1 | | | | | | | | | | | 2 | 7 | 1 | 1 | 3 | 5 | 4.0 | 32.0 |
| Group 2 | | | | 25 | 2 | | | | | 3 | | 1 | | 2 | 1 | 1 | 0.015 | 0.5 |
| TET | | | | | | | | | | | | | | | | | | |
| Group 1 | | | | | | | | | | | 1 | | | 5 | 24 | | 64 | 64 |
| Group 2 | | | | | | | | | | | | 2 | 19 | 3 | | | 16.0 | 64 |

[a] Abbreviations: PEN G, penicillin G; A-C, amoxicillin-clavulanic acid; CRO, ceftriaxone; CFX, cefixime; ERY, erythromycin; AZ, azithromycin; CIP, ciprofloxacin; OFX, ofloxacin; TMP-SMX, trimethoprim-sulfamethoxazole (1:19); TET, tetracycline; β-Lac+, β-lactamase positive; β-Lac−, β-lactamase negative.

[b] Group 1 isolates, isolates that possessed a 1.8-MDa plasmid in addition to other plasmids (see Table 1).

[c] Groups 2 isolates, isolates that possessed no plasmid or the following plasmids: 2.8, 3.2, 4.4, and 5.7 MDa; 2.8, 3.2, and 5.7 MDa; 2.8, 3.2, 5.7, and 7.4 MDa; 2.8, 3.2, and 7.4 MDa; 3.2 MDa only, 4.4 and 5.7 MDa; and 5.7 MDa only (see Table 1).

[d] MIC refers to the TMP concentration.

The results of the present study further support those of previous in vitro studies of erythromycin and ceftriaxone activity, which demonstrated that these agents are most active against isolates of *H. ducreyi* and support the use of erythromycin and ceftriaxone for the treatment of chancroid in the United States. Furthermore, these data and previous in vitro studies suggest that A-C and TMP-SMX should no longer be recommended for the treatment of chancroid in the United States unless continuous laboratory surveillance is undertaken to monitor susceptibilities to these agents. All of the newer agents tested were active against *H. ducreyi*; ceftriaxone was more active than cefixime, and azithromycin was more active than erythromycin. Although quinolones were usually active against *H. ducreyi* in the present study, one isolate was more resistant to them. Therefore, caution should be exercised when advocating the widespread use of quinolones for the treatment of chancroid, unless the susceptibilities of clinical isolates are routinely monitored for resistance. In the present study, as in another recent study (10), we identified the resistance phenotypes of *H. ducreyi*. These resistance phenotypes can be used to screen currently recommended therapeutic agents and newer agents for the treatment of chancroid.

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