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Comparison of the In Vitro Activities of Various Parenteral and Oral Antimicrobial Agents against Endemic *Haemophilus ducreyi*

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The in vitro susceptibilities to various antimicrobial agents of 100 strains of β-lactamase-producing *Haemophilus ducreyi* recently isolated from patients in New Orleans, La., were determined by an agar dilution method. All strains were highly susceptible to ceftizoxime, ceftriaxone, ceftazidime, azithromycin, erythromycin, ciprofloxacin, and sparfloxacin. β-Lactam–β-lactamase inhibitor combinations were less active, but all strains were susceptible to them. Doxycycline exhibited the poorest activity, and the rate of resistance to doxycycline varied depending on the time after inoculation that the MIC was determined.

Haemophilus ducreyi is the etiologic agent of chancroid, an important cause of genital ulcers worldwide. The disease is characterized by the development of single or multiple ulcerative lesions on the genitalia and may cause extensive destruction to the surrounding tissue. The infectious process often extends to the inguinal lymph nodes, resulting in a painful lymphadenitis, which may progress to bubo formation with suppuration. The latter may occur despite effective antimicrobial therapy in some cases. The major public health concern over chancroid is that studies in Africa have shown that the disease is a significant cofactor in the transmission of the human immunodeficiency virus (14).

Early in the antibiotic era, *H. ducreyi* was susceptible to almost all available drugs. However, increasing resistance has been documented among isolates from around the world. Most isolates are now β-lactamase producers and are also resistant to tetracycline (11). Increasing resistance to the sulfonamides, including trimethoprim-sulfamethoxazole, has been noted as well (12). Therefore, continued surveillance for the development of new resistance patterns among *H. ducreyi* strains is important. In the present study we compared the in vitro activities of several parenteral and oral antimicrobial agents against strains of *H. ducreyi* recently isolated from patients with chancroid in New Orleans, La., where a resurgence of this disease was first observed late in 1988.

One hundred clinical isolates of *H. ducreyi* were collected from the genital ulcers of patients attending a local sexually transmitted disease clinic. Swabs from the lesions were plated onto (i) heart infusion agar (Difco) supplemented with 5% defibrinated rabbit blood–5% fetal bovine serum–1% IsoVitaleX–3 μg of vancomycin per ml and (ii) GC II agar (BBL) supplemented with 1% bovine hemoglobin–5% fetal bovine serum–1% IsoVitaleX–3 μg of vancomycin per ml. Culture plates were streaked for organism isolation, incubated at 33°C in 6% CO_2 in a humidified chamber, and read daily for 7 days for the growth of typical waxy, yellow colonies. Typical colonies were subcultured onto chocolate II agar (BBL) for further identification. Identification of the isolates was based on the following scheme recommended by the Centers for Disease Control and Prevention (CDC) gram-negative bacillus; positive tests with oxidase, alkaline phosphatase, and nitrate reductase; and negative catalase and fluorescence for the porphyrin test with δ-aminolevulinic acid (10a). In addition, 20 randomly selected strains identified by this scheme in our laboratory were confirmed as *H. ducreyi* by CDC. Isolates were stored in Columbia broth (BBL) with 15% glycerol and 2% fetal bovine serum at −70°C until testing. The following antimicrobial agents were provided by the indicated manufacturers: ceftizoxime (Fujisawa Pharmaceuticals, Deerfield, Ill.), ceftriaxone (Roche Laboratories, Nutley, N.J.), ceftazidime (Glaxo Pharmaceuticals, Research Triangle Park, N.C.), ampicillin, cefoperazone, doxycycline, azithromycin, and sulbactam (Pfizer Laboratories, New York, N.Y.), ticarcillin, amoxicillin, and clavulanate (SmithKline Beechan, Philadelphia, Pa.), ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.), sparfloxacin (Park-Davis, Ann Arbor, Mich.), and erythromycin (Abbott Laboratories, Chicago, Ill.). Each agent was stored desiccated at −20°C until use.

Susceptibility testing of each isolate was performed by an agar dilution method as recommended by the National Committee for Clinical Laboratory Standards (9), with the exception of the test medium and inoculum size. The test medium consisted of GC II agar (BBL) supplemented with 1% bovine hemoglobin–1% IsoVitaleX–5% fetal bovine serum. Serial twofold dilutions of each antimicrobial agent within a twofold dilution range of 0.016 to 64 μg/ml were prepared in melted and cooled test medium and were poured into 100-by-100-mm petri dishes. When combining amoxicillin with clavulanate and ampicillin with sulbactam, a 2:1 ratio was used. A constant clavulanate concentration of 2 μg/ml was used when testing ticarcillin. The inoculum was prepared by suspending each isolate into 5 ml of tryptic soy broth equivalent to a 0.5 McFarland nephelometer standard, diluting the later 1:3 in tryptic soy broth, and placing 1 ml in a multiwell reservoir. By using a Steers replicating device, the antimicrobial agent-containing agar plates were stamped, giving a final inoculum of size of 1×10^5 to 2×10^5 CFU per spot. After the inoculum spot was absorbed, all plates were incubated at 33°C in 6% CO_2 and were read at 24 and 48 h. The MIC was defined as the lowest concentration of each antimicrobial agent (or combination) which completely inhibited the growth of the test isolate. Each susceptibility run included the following quality control organisms: *Esche-*

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TABLE 1. In vitro activities of various antimicrobial agents against 100 strains of *H. ducreyi*

Antimicrobial agent	MIC (μg/ml)[a]						% Susceptible[c]
	Mode		50%		90%		
	24 h	48 h	24 h	48 h	24 h	48 h	
Parenteral							
Ceftizoxime (≤8)[b]	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	100
Ceftriaxone (≤8)	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	100
Ceftazidime (≤8)	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	100
Ampicillin-sulbactam (≤8)	4	4	2	4	4	4	100
Cefoperazone-sulbactam (≤16)	0.5	1	0.25	1	1	2	100
Ticarcillin-clavulanate (≤16)	1	2	1	2	2	2	100
Oral							
Azithromycin (≤2)	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	100
Erythromycin (≤0.5)	≤0.03	≤0.03	≤0.03	≤0.03	≤0.06	≤0.06	100
Ciprofloxacin (≤1)	≤0.02	≤0.02	≤0.02	≤0.02	≤0.02	≤0.02	100
Sparfloxacin (≤1)	≤0.02	≤0.02	≤0.02	≤0.02	≤0.02	≤0.02	100
Doxycycline (≤4)	4	8	4	8	8	16	79/17
Amoxicillin-clavulanate (≤8)	1	2	1	2	2	4	100

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] Values in parentheses indicate the MICs which are considered susceptible. These values are based on the manufacturer's recommendations (2) or those of the National Committee for Clinical Laboratory Standards (9).
[c] Susceptibility at 24 h/48 h. A single value indicates no change in the susceptibility rate from 24 to 48 h.

richia coli ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *H. ducreyi* CIP542 (Pasteur Institute). β-Lactamase production was detected by use of a nitrocefin test (Cefinase; BBL).

Table 1 compares the in vitro activities of the various antimicrobial agents against the *H. ducreyi* strains. All three of the expanded-spectrum cephalosporins were highly active against these strains. Bower et al. (4) have shown similar in vitro results for both ceftriaxone and ceftazidime and excellent clinical efficacy of single-dose ceftriaxone administered intramuscularly in the treatment of chancroid. Our results with ceftizoxime suggest that this agent could be as effective clinically as ceftriaxone on the basis of its in vitro activity.

All of the test isolates were β-lactamase producers. β-Lactamase-producing strains of *H. ducreyi* have been shown to be resistant to penicillin and ampicillin (2). Although not as active as the expanded-spectrum cephalosporins, the β-lactam–β-lactamase inhibitor combinations showed good activity. All combinations had comparable activities, and no resistant strains were detected. Other investigators (7, 10) have reported synergistic activity between amoxicillin and clavulanate against β-lactamase-producing strains of *H. ducreyi*.

For the macrolides, the test strains were highly susceptible to both azithromycin and erythromycin. Slaney et al. (13) have previously reported that azithromycin has good activity against *H. ducreyi* strains originating from various parts of the world. Other investigators (2) have previously reported the good activity of erythromycin. The fluoroquinolones ciprofloxacin and sparfloxacin were highly active against all strains. Bodhidatta et al. (3) also reported results for ciprofloxacin similar to ours and showed excellent clinical results in the treatment of chancroid with ciprofloxacin administered orally. A review of susceptibility studies of strains of *H. ducreyi* from outside the United States by Dangor et al. (6) indicated results similar to those for our strains with regard to ceftriaxone, ceftazidime, erythromycin, ciprofloxacin, doxycycline, and amoxicillin-clavulanate.

Doxycycline was the only antimicrobial agent tested that showed an appreciable shift in the resistance rates between the readings at 24 and 48 h by using a susceptibility cutoff value of ≤4 μg/ml. At 24 h, the MICs for 79 strains were ≤4 μg/ml, while the MICs for 21 strains were ≥8 μg/ml. At 48 h, the MICs for 17 strains were ≤4 μg/ml and the MICs for 83 strains were ≥8 μg/ml. Bilgeri et al. (2) reported that for some *H. ducreyi* strains, doxycycline MICs were elevated at 24 h; however, they did not perform a reading at 48 h. Clinical resistance to tetracycline has been recognized since the early 1970s (1, 8). The reading of MICs of doxycycline at 48 h appears to be more predictive of clinical outcome. Modest upward shifts in the MIC profiles for several of the other drugs tested in the present study were noted at 48 h, but in all of these cases the MICs were below the breakpoint, so there appeared to be no benefit in delaying the MIC reading until 48 h. The present study showed that many orally and parenterally administered antimicrobial agents possess in vitro activity against β-lactamase-producing *H. ducreyi* strains isolated from November 1988 through November 1991 in New Orleans. Overall, the isolates were highly susceptible to expanded-spectrum cephalosporins, fluoroquinolones, and macrolides. β-Lactam–β-lactamase inhibitor combinations also showed good activity, while doxycycline was virtually inactive. The current recommendations for treating chancroid in the United States, as summarized in CDC guidelines in 1989 (5), include a single-dose regimen of ceftriaxone and multiple-dose regimens of erythromycin, ciprofloxacin, and ampicillin-clavulanic acid. Thus, our in vitro testing appears to be a valid predictor of in vivo efficacy with these antimicrobial agents.

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