ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1982, p. 686–688
0066-4804/82/100686-03$02.00/0
Vol. 22, No. 4

NOTES

Antimicrobial Susceptibility of 103 Strains of *Haemophilus ducreyi* Isolated in Johannesburg

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Received 16 June 1982/Accepted 28 June 1982

Of 103 strains of *Haemophilus ducreyi* isolated in Johannesburg, 96 produced β-lactamase and were resistant to penicillin and ampicillin. Most strains showed resistance to tetracycline, sulfisoxazole, and sulfamethoxazole. All isolates were susceptible to rifampin, erythromycin, and cefoxitin, moderately susceptible to trimethoprim-sulfamethoxazole (1:19) and minocycline, and somewhat less susceptible to doxycycline.

Chancroid, considered a rare disease in temperate climates, has recently been reported with increasing frequency in countries far removed from the tropics, such as Canada (6) and Greenland (10). Travelers to endemic regions may spread chancroid refractory to conventional therapy to their countries of origin. Strains of *Haemophilus ducreyi* isolated in Kenya (14) are more resistant than those from Canada (6), and many treatment failures using conventional therapy with sulfisoxazole or tetracycline have been reported in Vietnam (8, 12). Knowledge of the susceptibility patterns of *H. ducreyi* on a regional basis would therefore assist in the rational treatment of imported cases of the disease.

In South Africa, chancroid is endemic in some regions, including cities. We report the susceptibilities of 103 *H. ducreyi* isolates from patients in Johannesburg to 13 antimicrobial agents.

H. ducreyi strains were isolated from patients with genital ulceration in Johannesburg during 1980, using a modification of the method described by Hammond et al. (5). Swabs were inoculated onto agar plates consisting of Mueller-Hinton agar base (BBL Microbiology Systems, Cockeysville, Md.), 5% sterile horse blood heated to 75°C, 1% IsoVitaleX (BBL), and 3 μg of vancomycin per ml (Eli Lilly & Co., Indianapolis, Ind.) and incubated at 35°C for 48 h in a 5% CO_2 atmosphere with high humidity. The isolates were identified by the criteria of Kilian (9). *H. ducreyi* strains were lyophilized after suspension in 2 ml quantities of supporting fluid containing 1.7% (wt/vol) Neopeptone (Difco Laboratories, Detroit, Mich.), 3.3% (wt/vol) sucrose and 33.3% (vol/vol) horse serum. Reference powders of penicillin G (Glaxo Laboratories, Ltd., Greenford, Middlesex, England), ampicillin (Beecham Research Laboratories, Betchworth, England), cephaloridine (Eli Lilly & Co.), cefoxitin (Merck Sharp & Dohme, West Point, Pa.), tetracycline hydrochloride (The Upjohn Co., Kalamazoo, Mich.), doxycycline (Pfizer Inc., New York, N.Y.), minocycline (Lederle Laboratories, Pearl River, N.Y.), erythromycin base (Abbott Laboratories, North Chicago, Ill.), rifampin (Gruppo-Lepetit, Milan, Italy) and sulfisoxazole, sulfamethoxazole, trimethoprim, and trimethoprim-sulfamethoxazole (Hoffmann La Roche Inc., Nutley, N.J.) were used.

Minimal inhibitory concentrations (MICs) were determined by using an agar plate dilution technique. Reference strains of *H. ducreyi* ATCC 27722 and *Staphylococcus aureus* ATCC 25923 were included as controls. Heavy inocula of low-passage 48-h cultures of *H. ducreyi* and 24-h cultures of *S. aureus* were transferred from Mueller-Hinton broth (Difco) without IsoVitaleX. Suspensions of *H. ducreyi* were briefly dispersed with a Vortex mixer and coarse particles allowed to sediment for 15 min. The bacterial concentrations of the resulting supernatant were adjusted to approximately 10^8 colony-forming units per ml by comparison with a broth standard of *H. ducreyi*. *S. aureus* cultures were diluted to approximately 10^6 colony-forming units per ml. We used a multipoint inoculator (Denley Instruments, Sussex, England), which delivers 1-μl volumes, resulting in inocula of 10^5

TABLE 1. Susceptibility of 103 Johannesburg strains of *H. ducreyi* to 13 antimicrobial agents

	50% MIC (μg/ml)	90% MIC (μg/ml)	Range (μg/ml)
Penicillin G	128	>128	0.06–>128
Ampicillin	128	>128	0.12–>128
Cephaloridine	4.0	8.0	1.0–16
Cefoxitin	1.0	2.0	0.5–2.0
Tetracycline	8.0	16	0.25–32
Doxycycline	1.0	4.0	0.12–8.0
Minocycline	0.5	2.0	0.008–4.0
Erythromycin	0.008	0.03	0.0005–0.06
Rifampin	0.004	0.008	0.0001–1.0
Sulfisoxazole[a]	128	>128	8.0–>128
Sulfamethoxazole[a]	64	>128	4.0–>128
Trimethoprim[a]	4.0	16	0.5–32
Trimethoprim-sulfamethoxazole[a,b]	1.0	2.0	0.12–4.0

[a] The sulfonamides, trimethoprim, and trimethoprim-sulfamethoxazole were incorporated in enriched gonococcal agar base (6), whereas dilutions of all other antimicrobial agents were incorporated in Mueller-Hinton IsoVitaleX chocolate agar.

[b] Total trimethoprim-sulfamethoxazole in a ratio of 1:19.

and 10^3 colony-forming units for *H. ducreyi* and *S. aureus* strains, respectively. The MICs were performed in duplicate on 103 local isolates, and *H. ducreyi* ATCC 27722 and *S. aureus* ATCC 25923 were included as control organisms in all tests. Twofold serial dilutions of the drugs tested were made in the appropriate agar base covering a range of concentrations from 0.0001 to 128 μg/ml. All plates were examined after 24 h of incubation, and the MIC was taken as the lowest drug concentration allowing growth of three colonies or less.

β-Lactamase production was demonstrated by the chromogenic cephalosporin degradation method (15).

Results are summarized in Table 1. The MICs of *S. aureus* ATCC 25923 (not recorded) were within the expected ranges.

Resistance to penicillin and ampicillin was the result of β-lactamase production. Ninety-six isolates (93%) produced this enzyme, including eight isolates with relatively low MICs of 4 to 16 μg/ml. None of the β-lactamase-negative strains had MICs over 0.5 μg/ml.

With the advent of efficient media for the isolation (5) and susceptibility testing of *H. ducreyi* (6), more reliable studies have become possible. Hammond et al. (6) found 19 strains from Winnipeg, Canada, to be susceptible to rifampin, chloramphenicol, sulfisoxazole, and nalidixic acid, three isolates being resistant to penicillin and ampicillin as a result of a TEM-type β-lactamase (1, 11). Only one strain, also β-lactamase producing, was resistant to both tetracycline and doxycycline. South African and Kenyan strains (H. Nsanze, personal communication) are resistant to ampicillin, tetracycline, and sulfonamides, but susceptible to erythromycin, rosaramicin, and newer cephalosporins. The Johannesburg isolates were also highly susceptible to rifampin and moderately susceptible to minocycline and to trimethoprim-sulfamethoxazole.

A possible explanation for the high prevalence of β-lactamase-positive strains in Johannesburg is the extensive use of penicillin in the treatment of genital ulcers in this city irrespective of etiology. Of 96 β-lactamase producing strains, 11 had MICs of 4 or 8 μg/ml against ampicillin, whereas 85 had MICs of ≧16 μg/ml and 56 of these had MICs of ≧128 μg/ml. Relatively low MICs in the presence of β-lactamase production are known to occur, e.g., in *Haemophilus influenzae* (13), and a permeability barrier or cryptocity may be responsible (16). The β-lactamase produced by South African strains of *H. ducreyi* is mediated by plasmids which can be transmitted by conjugation (18) and which could have a common ancestry with the Kenyan strains.

Treatment with the highly recommended sulfisoxazole and tetracycline (17) appears to be inappropriate for South African patients. In Kenya, only 36% of patients responded to tetracycline and 77% responded to sulfadimidine (14). Therapy with both sulfisoxazole and tetracycline resulted in frequent failures in Vietnam (12). The use of minocycline and possibly doxycycline may also be contemplated, but single-dose therapy with doxycycline proved to be unsuccessful in Canada as did a 1-week course of sulfisoxazole, despite these isolates being fully susceptible to these agents (7). However, a patient in Sheffield who failed to respond to sulfadimidine treatment was cured with doxycycline (4).

Trimethoprim-sulfamethoxazole should give a

good therapeutic response, since high concentrations of trimethoprim are achieved in vaginal fluid (19), although the 1:19 ratio may not apply in these secretions. However, tissue levels may be more important, and good results have been achieved with therapy using this combination in Johannesburg (H. J. Koornhof, R. C. Ballard, M. O. Duncan, Y. R. Bilgeri, and G. Fehler, Curr. Chemother. Immunother., in press). Treatment with rifampin in combination with another agent to which *H. ducreyi* is susceptible may also prove to be highly efficient.

Feltham et al. (3) showed that rosaramicin, erythromycin, and clindamycin were highly active against *H. ducreyi*. Indeed, good results have been obtained in South Africa with erythromycin and rosaramicin therapy (unpublished data), and patients who had contracted chancroid in Korea responded excellently to erythromycin (2).

Based on in vitro activity coupled with considerations of toxicity and safety, erythromycin and rosaramicin appear to be the best prospects for further clinical evaluation, but the new cephalosporins also deserve consideration.

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