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Emergence of *Haemophilus ducreyi* Resistance to Trimethoprim-Sulfamethoxazole in Rwanda

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The in vitro susceptibilities of 112 clinical isolates of *Haemophilus ducreyi* to six antimicrobial agents were determined. These isolates were obtained in Kigali, Rwanda, during three studies on genital ulcer disease performed in 1986 (18 isolates), 1988 (23 isolates), and 1991 (71 isolates). All *H. ducreyi* isolates were susceptible to azithromycin, ceftriaxone, ciprofloxacin, and erythromycin; all isolates obtained in 1986 were also susceptible to trimethoprim and to the combination trimethoprim-sulfamethoxazole. In contrast, 39 and 9% of the isolates obtained in 1988 and 59 and 48% of the isolates obtained in 1991 were resistant to trimethoprim (MIC, ≥4.0 mg/liter) and trimethoprim-sulfamethoxazole (MIC, ≥4.0/76 mg/liter), respectively. These data indicate that trimethoprim-sulfamethoxazole can no longer be recommended for use in the treatment of chancroid in Rwanda, and possibly elsewhere in Africa.

Chancroid, which is caused by *Haemophilus ducreyi*, is a common cause of genital ulcer disease (GUD) in many developing countries. The current primary treatment regimen recommended by the World Health Organization is erythromycin, with ceftriaxone, ciprofloxacin, spectinomycin, and trimethoprim (TMP)-sulfamethoxazole (SMZ) used as alternative recommended regimens (14). Single-dose azithromycin therapy appears to be another promising alternative. Poor treatment outcomes with TMP-SMZ were observed in Asia many years ago and in East Africa more recently (9, 12).

Current methods of GUD diagnosis, including the isolation of *H. ducreyi*, are beyond the capacities of most settings in developing countries. Generally, patients with suspected cases of *H. ducreyi* infection are treated empirically as part of GUD case management. However, the therapeutic efficacy of recommended treatment regimens for GUD may change over time as a consequence of an increase in the level of resistance of local *H. ducreyi* strains to antimicrobial agents. Therefore, periodic surveillance of the clinical efficacy of treatment, the etiology of GUD, and in vitro antimicrobial susceptibilities of local *H. ducreyi* isolates is recommended.

TMP-SMZ has been the treatment of choice for chancroid in Rwanda for a long time, but treatment failures have recently been increasingly reported in the country. The present study was undertaken to determine the in vitro susceptibilities to recommended drugs of clinical isolates of *H. ducreyi* obtained between 1986 and 1991 in Kigali, which is the capital of Rwanda.

Between 1986 and 1992, 112 clinical isolates of *H. ducreyi* were collected. They were obtained at one clinic during three GUD studies performed in 1986 (18 isolates), 1988 (23 isolates), and 1991 (71 isolates). All isolates were obtained prior to treatment, and only patients who had not received antimicrobial therapy were included in the studies. The male-to-female ratio of the patients was 2:1. The human immunodeficiency virus seroprevalence increased from 59% in 1986 to 71% in 1991. The diagnostic findings of the first study were published previously (3). By using an agar dilution technique, the MICs of azithromycin, ceftriaxone, ciprofloxacin, erythromycin, TMP, and TMP-SMZ were determined. Antimicrobial agents were obtained as standard powders from different pharmaceutical companies. Serial twofold dilutions of the antimicrobial agents were added to the test medium, which consisted of GC agar (Difco Laboratories, Detroit, Mich.), 1% hemoglobin (Becton Dickinson, Cockeysville, Md.), 5% fetal bovine serum, 0.1% glucose, 0.01% L-glutamine, and 0.025% L-cysteine hydrochloride. The growth from 48-h *H. ducreyi* cultures on GC agar (Difco) enriched with 1% hemoglobin (Becton Dickinson)–1% IsoVitaleX (Becton Dickinson)–5% fetal bovine serum was suspended in Mueller-Hinton broth (Difco), vortexed, and allowed to sediment for 20 min. The supernatant was transferred to another tube and was adjusted to a concentration of 10^8 bacteria per ml by comparison with a 0.5 McFarland standard. With a multipoint replicator, inocula of 10^5 CFU were spotted onto the test medium. MIC results were read after incubation of the test plates for 48 h at 35°C in 5% CO_2 atmosphere.

The MIC ranges and the MICs for 50 and 90% of isolates tested are given in Table 1. All *H. ducreyi* isolates were susceptible to azithromycin, ceftriaxone, ciprofloxacin, and erythromycin. Overall, 51 (46%) isolates were resistant to TMP (MIC, ≥ 4.0 mg/liter) and 36 (32%) isolates were resistant to TMP-SMZ (MIC, ≥4.0/76 mg/liter). By examining the MICs over time, no differences in susceptibilities to azithromycin, ceftriaxone, ciprofloxacin, and erythromycin were observed. However, for TMP and TMP-SMZ a significant increase in the MICs occurred over time. The number and percentage of resistant *H. ducreyi* isolates are given in Table 2. Neither gender nor the human immunodeficiency virus infection status of patients was associated with this resistance pattern. SMZ alone was tested on 30 isolates only; 14 of 18 isolates obtained in 1986 and 10 of 12 isolates obtained in 1988 were resistant to SMZ (MIC, ≥ 128 mg/liter), and SMZ resistance was not associated with TMP-SMZ resistance.

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TABLE 1. Antimicrobial susceptibilities of 112 *H. ducreyi* isolates obtained in Rwanda between 1986 and 1991

Antimicrobial agent	MIC (mg/liter)[a]		
	Range	50%	90%
Azithromycin	0.002–0.125	0.004	0.008
Ceftriaxone	0.001–0.06	0.001	0.002
Ciprofloxacin	0.002–0.06	0.015	0.015
Erythromycin	0.004–0.25	0.015	0.03
TMP	0.06–32	2.0	32
TMP-SMZ (1/19)	0.6–320	20	320

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

The activities of azithromycin, ceftriaxone, ciprofloxacin, and erythromycin against *H. ducreyi* isolates observed in the present study were very similar to those reported elsewhere (1, 2, 5, 7–9). The changing susceptibility patterns for TMP and TMP-SMZ observed between 1986 and 1991 suggest that this type of resistance has occurred recently. Most *H. ducreyi* isolates in Africa and Asia are resistant to SMZ, although many SMZ-resistant strains have remained susceptible to the combination TMP-SMZ (10, 11). TMP-SMZ-resistant organisms are generally resistant to both components of the mixture. In the present study, all 36 TMP-SMZ-resistant isolates were resistant to TMP (MIC, $\geq$ 4.0 mg/liter), and 15 of the 51 TMP-resistant isolates (29%) were still susceptible to the combination TMP-SMZ (MIC, $\leq$ 2.0/38 mg/liter). The data obtained in the present study strongly suggest that the appearance of TMP-SMZ-resistant *H. ducreyi* isolates in Rwanda is a consequence of the recent emergence of TMP resistance. Resistance to TMP may be due to several mechanisms. It is often due to plasmid-mediated dihydrofolate reductases that may become incorporated into the chromosome via transposons. It may also be due to the overproduction of dihydrofolate reductase, changes in cell permeability, or the presence of bacterial mutants which are intrinsically resistant to TMP because they depend on exogenous thymine and thymidine for growth (6).

Results of our in vitro antimicrobial susceptibility study suggests that TMP-SMZ should no longer be recommended for use in the treatment of chancroid in Rwanda, and possibly elsewhere in central Africa. Erythromycin is now recommended by the World Health Organization (14) and the Centers for Disease Control and Prevention for the treatment of chancroid (4). Single-dose treatment with ceftriaxone, ciprofloxacin, and potentially, azythromycin may offer attractive alternatives, although studies in Kenya showed that single-dose ceftriaxone and single-dose fleroxacin are no longer reliable or effective therapies in that country (9, 13).

TABLE 2. TMP and TMP-SMZ resistance in *H. ducreyi* isolates over time

Year	No. of resistant isolates/total no. of isolates (%)[a]	
	TMP (MIC, $\geq$ 4.0 mg/liter)	TMP-SMZ (MIC, $\geq$ 4.0/76 mg/liter)
1986	0/18 (0)	0/18 (0)
1988	9/23 (39)	2/23 (9)
1991	42/71 (59)	34/71 (48)

[a] χ^2 for trend, $P < 0.0001$ for both antimicrobial agents.

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