Antimicrobial Susceptibilities of Southern African Isolates of Haemophilus ducreyi

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We determined the antimicrobial susceptibilities of 122 recent clinical isolates of Haemophilus ducreyi to 24 antimicrobial agents. All isolates produced β-lactamase and were resistant to penicillins. The majority of strains were also resistant to tetracycline, doxycycline, and sulfamethoxazole. All isolates were susceptible to macrolides, quinolones, extended-spectrum cephalosporins, spectinomycin, rifampin, and amoxycillin-clavulanate. Reduced susceptibility to minocycline, co-trimoxazole, and kanamycin was noted. Chloramphenicol and thiampenicol resistance was noted for the first time among southern African strains.

Chancroid is endemic in many tropical and subtropical countries, and occasional reports have appeared documenting the occurrence of the disease in more temperate climates (7). In Africa, chancroid is the commonest cause of genital ulcer disease, accounting for 80% of cases in Nairobi (15), 42% in Swaziland (13), and 39% in Zimbabwe (11). Previous studies in southern Africa have shown that chancroid is the major cause of genital ulceration in black men (2). The present study was undertaken to determine the current antimicrobial susceptibilities of southern African strains of Haemophilus ducreyi and to compare this with data published from this department in 1982 (1).

One hundred and twenty-two strains of H. ducreyi were isolated from migrant miners in South Africa with genital ulcer disease. In 72% of the cases, chancroid was locally acquired in the Johannesburg and Carletonville areas, and in the remaining 28%, infection was acquired in Lesotho, Botswana, Swaziland, and Transkei. Swabs from ulcers were inoculated onto Mueller-Hinton agar base (BBL Microbiology Systems, Cockeysville, Md.), supplemented with 5% sterile horse blood heated to 75°C, 1% IsoVitaleX (BBL), and 3 μg of vancomycin per ml as well as enriched gonococcal agar base (GIBCO Diagnostics, Madison, Wis.) (6), and incubated at 35°C for 48 h in a 5% CO₂ atmosphere with high humidity. H. ducreyi strains were identified by the criteria of Kilian (9). A heavy inoculum of each strain was stored in skim milk with glycerol in liquid nitrogen before testing.

Reference powders of the following agents were used to test antimicrobial susceptibility: penicillin G (Glaxo Pharmaceuticals, Ltd., Greenford, United Kingdom); Augmentin (amoxycillin-clavulanic acid) (Beecham Laboratories, Bristol, Tenn.); tetracycline and spectinomycin (The Upjohn Co., Kalamazoo, Mich.); doxycycline (Pfizer Inc., New York, N.Y.); minocycline (Lederle Laboratories, Pearl River, N.Y.); erythromycin base; difloxacin (A-56619) and A-56268 (Abbott Laboratories, North Chicago, Ill.); chloramphenicol and thiampenicol (Zambon); ciprofloxacin (Bayer Pharmaceuticals); roxacin (Winthrop Laboratories, Div. Sterling Drug Inc., New York, N.Y.); ceftriaxone and RO 19-5247 (ceftetrame) (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.); kanamycin (Bristol); rifampin (Gruppo Lepetit, Milan); trimethoprim, sulfamethoxazole, and trimethoprim-sulfamethoxazole (1:19) (Hoffmann-La Roche).

MICs were determined by using the agar dilution method. Reference strains of H. ducreyi ATCC 27722 and CIP 542 and Staphylococcus aureus ATCC 25923 were included as controls. Heavy inocula of low-passage cultures of H. ducreyi and 24-h cultures of S. aureus ATCC 25923 were made into Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) with 1% IsoVitaleX (BBL). The broth suspension was dispersed by ultrasonication at 6 μm for 15 s (Soniprep 150 MSE). The inoculum size of H. ducreyi was adjusted to approximately 10⁸ CFU/ml, and the S. aureus culture was diluted to yield approximately 10⁶ CFU/ml.

The agar dilution method was carried out on enriched gonococcal agar base (6) with a multipoint inoculator (Denley Instruments, Sussex, England) which delivered 1-μl volumes. This resulted in inocula of 10⁵ and 10⁶ CFU/ml for H. ducreyi and S. aureus strains, respectively. Plates were incubated for 48 h at 35°C in 5% CO₂ in a humid atmosphere. MICs were determined as the lowest concentrations yielding a growth of three or fewer colonies, except in tests for sulphonamides and trimethoprim, in which 80% inhibition of growth was regarded as the criterion for susceptibility. Isolates were examined for β-lactamase production by the chromogenic cephalosporin degradation method (16). The results are presented in Table 1. All strains produced β-lactamase, but the MIC for 50% of the strains tested (MIC₅₀) of penicillin (16 μg/ml) was somewhat lower than that previously reported for H. ducreyi in Johannesburg (1). All strains were resistant to amoxycillin (MIC₅₀, 64 μg/ml). MICs of amoxycillin were markedly reduced in the presence of clavulanic acid. MIC₅₀s of amoxycillin were 4, 2, and 1 μg/ml and MIC₉₀s were 64, 4, and 4 μg/ml at fixed concentrations of 1, 2, and 4 μg of clavulanic acid per ml, respectively. This indicates that synergism occurs between the two components and suggests that the combination should be effective in the treatment of chancroid (15). H. ducreyi strains isolated in Paris, Nairobi, and Bangkok proved to be very susceptible to several broad-spectrum cephalosporins (17, 19, 22). For southern African strains of H. ducreyi, ceftriaxone was demonstrated to be the most active of β-lactam antibiotics as well as an extended-spectrum cephalosporin, with MIC₉₀ of 0.002 to 0.008 μg/ml, with the next most active agents being cefotaxime and ceftetrame (RO 19-5247).

More than 50% of H. ducreyi strains isolated in Kenya,
South Africa, the Philippines, and Singapore have been reported to be resistant to tetracycline (1, 8). Southern African strains have also been found to be moderately resistant to minocycline (1). The present study shows that 10% of isolates are now resistant to minocycline (MIC, ≥4 µg/ml), and it is possible that minocycline resistance may become a clinically significant problem in the future. We have also demonstrated an increase in resistance of isolates to tetracycline and doxycycline, with MIC₉₀ values of 128 and 32 µg/ml, respectively. All our isolates were fully susceptible to spectinomycin. Clinical trials have demonstrated reasonable good clinical efficacy of spectinomycin, with cure rates of 83 and 94% after single intramuscular doses of 2 and 4 g, respectively (5, 14).

Various studies have shown that ciprofloxacin, rosoxacin, ofloxacin, and pefloxacin have excellent in vitro activity against H. ducreyi (21–23). Our strains were fully susceptible to ciprofloxacin, rosoxacin, and ofloxacin (A-56619), with MIC ranges of ≤0.004 to 0.125 µg/ml for each. The majority of isolates of H. ducreyi worldwide are considered to be susceptible to erythromycin. Erythromycin is extensively used in South Africa for the treatment of chancroid (3). All isolates remain susceptible to erythromycin and to the macrolide A-56268. In previous studies, African isolates of H. ducreyi were shown to be susceptible to chloramphenicol and thiamphenicol (12). In Zimbabwe, thiamphenicol has been successfully used for the treatment of chancroid (12).

In the present study, we documented for the first time the emergence of chloramphenicol and thiamphenicol resistance among Southern African strains of H. ducreyi. Preliminary investigations indicate that these strains produce the enzyme chloramphenicol acetyltransferase. Trimethoprim has been shown to be active against H. ducreyi isolated in Nairobi and Amsterdam (18, 20). In the present study, 14% of isolates remain fully resistant to trimethoprim, with a MIC₉₀ of 4 µg/ml and a MIC range of ≤0.125 to 16 µg/ml. This is somewhat less than that documented in 1982 (1). Bilgeri et al. previously reported that 50% of strains were resistant to sulfamethoxazole (1). This appears to be unchanged in the present study. The combination of trimethoprim-sulfamethoxazole has been used with complete clinical success for the treatment of chancroid in Africa (4, 10) and in Amsterdam (21). Our findings indicate that the majority of our isolates of H. ducreyi remain susceptible to this combination, although MICs in excess of 2 µg/ml were found for 13% of the strains. A changing pattern of antimicrobial susceptibility and resistance among Southern African isolates of H. ducreyi has emerged since the previous study of Bilgeri et al. in 1982. The reasons are not fully clear but may be related to the widespread use of certain antimicrobial agents such as tetracycline and chloramphenicol for the treatment of chancroid. Erythromycin remains the drug of choice for the treatment of H. ducreyi infections, although the broad-spectrum cephalosporins and quinolones may ultimately prove to be of equivalent efficacy.

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