Susceptibility of *Haemophilus ducreyi* to spectinomycin in vitro

S HAFIZ,* B M JONES,* G R KINGHORN† AND B I DUERDEN*

From the *Department of Medical Microbiology, University of Sheffield Medical School, and the †Department of Genitourinary Medicine, Royal Hallamshire Hospital, Sheffield

SUMMARY Using an agar dilution technique with standardised inocula prepared by ultrasonication, the minimum inhibitory concentrations (MICs) of spectinomycin were determined for 66 strains of *Haemophilus ducreyi*, eight of which were β lactamase producers. All strains were sensitive to concentrations of spectinomycin, which were well within the therapeutic ranges attained with normal dosage. The MIC\(_{50}\) and MIC\(_{90}\) were 8 mg/l, (range 0-007–16 mg/l).

**Introduction**

Previous studies of the antimicrobial sensitivity of *Haemophilus ducreyi*, which causes classical chancroid and also contributes to other forms of genital ulcerations, have shown varied results, particularly with aminoglycosides and β lactam antibiotics. Sng \(et\ al\) found that all 17 strains studied were sensitive to streptomycin, but Sturm and Zanen\(^2\) reported that 11 of 19 strains of *H ducreyi* were resistant to aminoglycosides. In a separate study in this department we found that all 36 strains tested were sensitive to gentamicin, tobramycin, and amikacin (unpublished data). Aminoglycosides have been widely used to treat cases of chancroid caused by β lactam resistant strains. Asin\(^4\) found that ulcers often relapsed and buboes progressed to suppuration during treatment with streptomycin, but both Marmar\(^5\) and Hart\(^6\) reported successful treatment with kanamycin of patients who had failed to respond to sulphonamide and tetracycline. Luders \(et\ al\)\(^7\), Bardasch\(^8\) and Morel \(et\ al\)\(^9\) each obtained successful results with streptomycin, kanamycin, or gentamicin. Rajan and Sng,\(^10\) however, observed that in Singapore 52% of chancroid cases treated with either streptomycin or kanamycin failed to respond, whereas before 1980 excellent results with either drug had invariably been obtained. They suggested that the increased use of aminoglycosides to treat β lactamase producing *Neisseria gonorrhoeae* may have led to changes in the sensitivity patterns of Asian *H ducreyi* strains. Spectinomycin is an aminocyclitol compound with the same mode of action as, but less toxic than, aminoglycosides; and it has become the preferred drug in many places for the treatment of β lactamase producing *N gonorrhoeae*. It would also be an appropriate agent for use in *H ducreyi* infections.

Until recently, the peculiarly cohesive nature of *H ducreyi*, which renders colonies impossible to emulsify, and the inability of the organism to grow in liquid media or on routine sensitivity test agar, have impeded accurate in vitro antimicrobial susceptibility determinations. We developed an ultrasonication method to prepare smooth standardised inocula of *H ducreyi* strains and used these in an agar dilution technique to determine the minimum inhibitory concentration (MIC) of spectinomycin for a series of strains.

**Materials and methods**

**HAEMOPHILUS DUCREYI STRAINS**

A total of 66 strains, which had been isolated from cases of classical chancroid and other forms of genital ulceration, were examined: 49 were isolated from patients attending the department of genitourinary medicine, Royal Hallamshire Hospital, Sheffield; four...
were reference strains from the National Collection of Type Cultures, Colindale, London; six were from Ghana (received from Dr RA Ronald), five from the USA (Drs H Hunter-Hansfield and JR Greenwood), one from Canada (Mr I MacLean) and one from Singapore (Dr EH Sng). All were identified according to the methods of Hafiz et al and were stored in liquid nitrogen at -130°C before testing.

PREPARATION OF INOCULA
Standardised suspensions for MIC tests were prepared by ultrasonication. Strains were grown on Sheffield medium for four to five days at 33°C in a humidified incubator with an atmosphere of 5% carbon dioxide. Several colonies were harvested into 2 ml sterile saline and dispersed into smooth suspension by ultrasonic treatment at 6 𝜇m for 10 seconds (Ultrasonic Disintegrator, MSE Ltd). This was diluted in saline to give an inoculum suspension of ≈ 10⁶ colony forming units (cfu)/ml by comparison with match opacity tubes (Wellcome Ltd) previously calibrated by having been compared with surface viable counts of sonicated suspensions.

DETERMINATIONS OF MINIMUM INHIBITORY CONCENTRATIONS
An agar dilution method was used with spectinomycin (Upjohn Ltd). A stock solution containing 320 mg/l was prepared in distilled water. Doubling dilutions were made and added to Sheffield medium to produce a set of plates containing a range of spectinomycin concentrations from 16-0-007 mg/l. A multipoint inoculator (Denley Instruments Ltd) was used to deliver 0-001 ml inocula (10⁴ cfu) to the surface of the plates containing antibiotic. Sheffield medium without antibiotic was also inoculated as a growth control. All were incubated at 33°C in a humidified incubator with an atmosphere of 5% carbon dioxide. Results were recorded after four days, and the MICs were taken as the lowest concentrations of spectinomycin that prevented growth.

Results
All 66 strains of H. ducreyi were sensitive to spectinomycin. The MIC range was 0-007-16 mg/l, with MIC<sub>50</sub> and MIC<sub>90</sub> values of 8 mg/l. The MIC values for the eight ß-lactamase producing strains were slightly lower than the values for all strains, range 0-007-8, MIC<sub>50</sub> 0-5 and MIC<sub>90</sub> 4 mg/l.

Discussion
The combination of the Sheffield medium for the growth of H. ducreyi and the sonication procedure for producing reproducible inocula provided a reliable method for determining the antimicrobial susceptibility of H. ducreyi. Our results show that H. ducreyi strains from various sources are susceptible to the aminocyclitol antibiotic spectinomycin, which seems to have more reliable activity against this organism than the related aminoglycosides. The MIC values of spectinomycin were well within the range of serum concentrations readily achieved by conventional dose regimens, and this antibiotic may have a useful role in the treatment of chancroid and other infections associated with H. ducreyi.

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