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Susceptibility of 40 *Haemophilus ducreyi* Strains to 34 Antimicrobial Products

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A study was performed to examine compounds that might improve the selectivity of the primary isolation medium for *Haemophilus ducreyi*. The susceptibility of 40 *H. ducreyi* strains to 34 antimicrobial agents, including 10 antibiotics, 3 quaternary ammonium compounds, 3 phenolic derivatives, 3 acridines, and 15 heavy metal compounds, was investigated by using an agar plate dilution technique. Results were compared with the susceptibilities of other gram-negative rods which may be contaminants on isolation media. The minimal inhibitory concentration range for colistin (16 to 128 μg/ml) indicated that this antibiotic might be of use as a selective agent. *H. ducreyi* was susceptible to spectinomycin (minimal inhibitory concentration range, 16 to 64 μg/ml), thiamphenicol (0.12 to 1 μg/ml), chloramphenicol (0.12 to 0.5 μg/ml), and streptomycin (4 to 32 μg/ml) and moderately susceptible to kanamycin (2 to 8 μg/ml). For the heavy metal compounds, a high susceptibility was seen with copper(II) chloride (2 to 8 μg/ml, corresponding to a concentration of 0.75 to 3 μg of Cu^{2+} ions per ml), sodium selenite (1 to 4 μg/ml, or 0.45 to 1.83 μg of Se^{-} ions per ml), and phenylmercury acetate (0.12 to 0.5 μg/ml). The minimal inhibitory concentrations of quaternary ammonium compounds, acridines, and phenolic derivatives were between 1 and 32 μg/ml, 8 and 32 μg/ml, and 8 and 250 μg/ml, respectively.

The isolation of *Haemophilus ducreyi* from clinical material is often difficult because of a lack of sensitivity and specificity of the primary isolation medium. Several diagnostic criteria have been proposed in the past, but most of these have a low accuracy or yield low recovery rates (1, 2, 6, 8). As a consequence, the diagnosis of chancroid is often based on the exclusion of other sexually transmitted diseases causing genital ulcers and on the clinical aspects of the lesions (5, 9). The isolation medium developed by Hammond et al. (7) and its subsequent modifications (3, 4, 10) contain vancomycin. While effectively inhibiting the gram-positive flora, this medium does not inhibit gram-negative rods which often contaminate isolation plates, particularly when vaginal or cervical specimens are cultured.

In this study, the activity of 34 antimicrobial agents, including 10 antibiotics, 3 quaternary ammonium and 15 heavy metal compounds, 3 acridines, and 3 phenolic derivatives was evaluated against 40 strains of *H. ducreyi*. Eighteen clinical isolates of *Enterobacteriaceae* were tested in the same way to define the relative susceptibilities to the same agent. The aim of the study was to select a compound that would enhance the selectivity of the isolation medium.

MATERIALS AND METHODS

Strains and media. Forty *H. ducreyi* strains, including isolates from Kenya (H. Nsanze), South Africa (R. C. Ballard), Canada (A. R. Ronald and W. Albritton), and The Netherlands (Sturm) and four strains from the Institut Pasteur (A75, A76, A77 and 542), were tested. All of the strains showed good growth on a solid medium consisting of Mueller Hinton agar (BBL Microbiology Systems), hemin (200 μg/ml) (BDH), glucose (0.1%, wt/vol) (Difco Laboratories), L-glutamine (0.01%, wt/vol) (E. Merck AG), cysteine hydrochloride (0.05%, wt/vol) (BDH), and bovine albumin fraction V (0.2%, wt/vol) (Armour), pH 7.2. This medium was also used for the agar dilution technique. Inocula were prepared in broth medium, i.e., medium with the same composition but without agar.

The 18 *Enterobacteriaceae* strains isolated from clinical specimens in Antwerp, Belgium, were *Escherichia coli, Salmonella paratyphi, Salmonella typhi, Morganella morganii, Klebsiella rhinoscleromatis, Klebsiella pneumoniae, Edwardsiella tarda, Serratia liquefaciens, Citrobacter intermedium, Citrobacter freudii, Proteus vulgaris* (two strains), *Shigella flexneri, Enterobacter agglomerans, Enterobacter cloacae, Serratia marcescens, Providencia alcalifaciens*, and *Yersinia enterocolitica*. These strains were tested on the same medium that was used for *H. ducreyi* to get comparable results. Inocula were prepared in brain heart infusion broth (Difco).

Antimicrobial compounds. The following compounds were tested: the antibiotics colistin (Roger Bellon), spectinomycin (activity, 66%; The Upjohn Co.), methenamine (Parke, Davis & Co.), cycloserine (Roche Diagnostics), novobiocin (Upjohn), streptomycin (Continental Pharma), nitrofurantoin (Gist-Brocades), kanamycin and chloramphenicol (Ministry of Health, Brussels), and thiamphenicol (Inpharzam-Zamlon); the phenolic compounds *p-tert*-amylphenol (Janssens Pharmaceuticals), resorcin (Merck), and *p*-hydroxybenzoic acid (Sigma Chemical Co.); the acridines acridine (Sigma), 3,6-diaminoacridine (Aldrich Chemical Co.), and 9-aminoacridine hydrochloride (Aldrich); the quaternary ammonium compounds cetylpyridinium chloride (Sigma), dodecyltrimethylammonium bromide (Sigma), and benzalkonium (Sigma); and the heavy metal compounds $NiCl_2 \cdot 6H_2O$ (Merck), $CoCl_2 \cdot 6H_2O$ (Merck), $FeCl_2 \cdot 4H_2O$ (Merck), $CdCl_2 \cdot H_2O$ (Merck), $SnCl_2 \cdot 2H_2O$ (Merck), $ZnCl_2$ (Merck), $CuCl_2 \cdot 2H_2O$ (Merck), $AgNO_3$ (Merck), $HgCl_2$ (Merck), sodium selenite (Riedel de Haen), potassium selenate (Riedel de Haen), phenylmercury acetate (Sigma), selenomethionine (Sigma), selenocystamine (Sigma), and lead acetate (Merck).

Agar dilution. Twofold dilutions of the antimicrobial compounds were incorporated in the agar medium. Overnight broth cultures of *H. ducreyi* containing 10^7 CFU/ml were used to inoculate these plates with a Steers Multipoint inoculator (Denley-Tech Ltd.), resulting in a final inoculum of 10^4 CFU per spot. Broth cultures of *H. ducreyi* were vigorously shaken for 30 s on a vortex mixer to break the chains of bacteria. Thus, clumping of *H. ducreyi* was greatly reduced. Overnight broth cultures of the *Enterobacteriaceae* were diluted to the same concentration. After every 10 test plates, plates without antimicrobial compound were inoculated to evaluate the quality of the inoculum. If a strain showed only minor growth on at least one of these control plates, it was excluded from final interpretation. Plates were incubated for 48 h at 35°C and 5% CO_2 in a humid atmosphere. The minimal inhibitory concentration (MIC) was taken as the lowest concentration allowing growth of three or fewer colonies.

RESULTS

Results of susceptibility tests are given in Tables 1 (antibiotics) and 2 (heavy metal compounds, phenolic derivatives, acridines, and quaternary ammonium compounds).

For the heavy metal compounds, the following three groups could be distinguished: one group showing MICs ranging from 256 to 1,000 μg/ml, a second group with values ranging from 16 to 128 μg/ml, and three compounds that showed the highest activity, namely, copper(II) chloride (MIC, 2 to 8 μg/ml), sodium selenite (1 to 4 μg/ml), and phenylmercury acetate (0.12 to 0.5 μg/ml). For the copper and selenium compounds, this activity corresponds to an ion concentration of 1 to 4 and 0.45 to 1.83 μg/ml, respectively. The MIC range for the acridine compounds was 8 to 32 μg/ml, as was the case for the quaternary ammonium compounds, with a slightly higher activity for benzalkonium (1 to 8 μg/ml). The MICs of the phenolic compounds ranged between 8 and 250 μg/ml, with the highest susceptibility being to *p-tert*-amylphenol (MIC, 8 to 32 μg/ml).

TABLE 1. Susceptibility of *H. ducreyi* strains to 10 antibiotics

Compound	No. of strains	MIC (μg/ml)		
		Range	50%	90%
Methenamine	32	64–250	250	250
Cycloserin	31	32–128	64	128
Colistine	29	16–128	64	128
Spectinomycin	34	16–64	32	32
Streptomycin	29	4–32	16	32
Kanamycin	29	2–8	4	8
Novobiocin	29	1–4	2	4
Nitrofurantoin	30	0.12–1	0.25	0.5
Thiamphenicol	29	0.12–1	0.25	0.25
Chloramphenicol	29	0.12–0.5	0.25	0.25

The 18 *Enterobacteriaceae* strains were tested with the same drug concentrations as *H. ducreyi*. Only colistin inhibited some of the strains at concentrations that were lower than those required for inhibition of *H. ducreyi* strains.

DISCUSSION

All of the compounds tested yielded MIC ranges covering a maximum of four dilution steps. Thus, *H. ducreyi* strains exhibited no great variability in their response to the inhibitory action of these compounds.

The in vitro activity of thiamphenicol (MIC, 0.12 to 1 μg/ml) and chloramphenicol (0.12 to 0.5 μg/ml) suggests a possible therapeutic use of these antibiotics. In a recent study in Zimbabwe (9), thiamphenicol was reported to provide an overall cure rate of 93.8%. However, only 59% of the subjects were cured after a single dose regimen, and 34.8% required a second dose. Furthermore, the isolation of *H. ducreyi* was not accomplished. *H. ducreyi* was also susceptible to streptomycin (MIC, 4 to 32 μg/ml), and prolonged courses of treatment with this antibiotic have been used successfully (5). The MIC range of spectinomycin was found to be 16-64 μg/ml. This is in agreement with the MIC range that was found in a recent study from Singapore (12), indicating that Asian strains show the same susceptibility to this antibiotic. Spectinomycin may, therefore, be an alternative therapy for chancroid.

Of all the 34 products tested, only colistin may enhance the selectivity of a primary isolation medium. The MICs of colistin for *H. ducreyi* (16 to 128 μg/ml) were higher than those for several *Enterobacteriaceae* strains which frequently overgrow primary isolation plates for *H. ducreyi*. The inhibited strains included *E. coli*, *S.*

TABLE 2. Susceptibility of *H. ducreyi* strains to 24 antimicrobial compounds

Compound	No. of strains	MIC (μg/ml)		
		Range	50%	90%
Selenomethionine	33	250–1,000	500	750
$FeCl_2 \cdot 4H_2O$	31	500–750	500	750
$SnCl_2 \cdot 2H_2O$	31	250–500	500	500
$CoCl_2 \cdot 6H_2O$	31	250–500	500	500
$NiCl_2 \cdot 6H_2O$	31	250–500	500	500
Potassium selenate	33	64–500	500	500
$AgNO_3$	31	32–128	128	128
$ZnCl_2$	31	32–64	32	32
$HgCl_2$	31	64	64	64
Lead acetate	33	16–128	64	64
Selenocystamine	30	16–128	64	64
$CdCl_2 \cdot H_2O$	31	16–32	32	32
$CuCl_2 \cdot 2H_2O$	31	2–8	8	8
Sodium selenite	32	1–4	4	4
Phenylmercury acetate	33	0.12–0.5	0.25	0.25
Acridine	33	8–32	32	32
9-Aminoacridine	32	16–32	32	32
3,6-Diaminoacridine	33	8–16	8	16
Cetylpyridinium chloride	32	8–32	16	32
Dodecyltrimethylammonium bromide	32	8–32	16	32
Benzalkonium	32	1–8	8	8
Resorcin	32	256	256	256
p-Hydroxybenzoic acid	32	64–256	128	128
p-tert-Amylphenol	32	8–32	16	32

paratyphi, S. typhi, K. rhinoscleromatis, K. pneumoniae, S. flexneri, E. agglomerans, E. cloacae, C. intermedium, and *C. freudii*. Unfortunately, colistin is inactive against *Proteus* sp., of which the swarming strains in particular cause difficulties in isolation. However, published MICs for *E. coli*, salmonellae, and shigellae range from 0.1 to 4 μg/ml (11). Colistin was used by Hammond et al. (7) at a concentration of 7.5 μg/ml and was found to be inhibitory for *H. ducreyi*. The concentration used in that study was only one dilution less than the MIC range we found. The evaluation of colistin as an inhibitory agent was also made during an epidemic in a defined urban society, probably involving one clone of *H. ducreyi* (7a). It may be useful to reevaluate various concentrations of colistin as a selective isolation agent in areas where chancroid is endemic.

H. ducreyi strains showed a high susceptibility to sodium selenite (MIC, 1 to 4 μg/ml) and copper(II) chloride (2 to 8 μg/ml). The MICs for acridines (8 to 32 μg/ml), quaternary ammonium compounds (1 to 32 μg/ml), and phenolic derivatives (8 to 250 μg/ml) were also considerably less than the concentrations used in common local antiseptics (13).

Other *Haemophilus* strains, including strains of *H. influenzae, H. avium, H. paraphrophilus, H. equigenitalis, H. pleuropneumoniae, H. parainfluenzae*, and *H. aegyptium*, were more resistant to copper and selenium, but they were even more susceptible to phenylmercury acetate than was *H. ducreyi* (unpublished data). All *H. ducreyi* strains were inhibited by 64 and 250 μg/ml of $HgCl_2$ and resorcine, respectively. This susceptibility pattern may be useful for the differentiation of *H. ducreyi* from other *Haemophilus* strains.

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