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Novel Plasmid Combinations in *Haemophilus ducreyi* Isolates from Thailand

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Thirty isolates of *Haemophilus ducreyi* collected in Thailand in 1984 were characterized by plasmid content. Three novel plasmids with estimated molecular masses of 1.8, 2.6, and 2.8 MDa were observed in 29 isolates, in addition to the 3.2-, 5.7-, and 7.0-MDa β-lactamase and 4.4-MDa sulfonamide resistance plasmids. At least three of the seven plasmids were observed in each of the 29 isolates. The number and diversity of plasmids observed in these isolates of *H. ducreyi* distinguish them from strains previously described.

Chancroid is a common sexually transmitted disease in Thailand (3) and in many other developing countries (8, 11). Effective methods of controlling chancroid depend on understanding the epidemiology of the disease. Various methods have been examined for their potential utility as epidemiological tools for studying the distribution of strains of *Haemophilus ducreyi*. Such methods include lectin typing (5), outer membrane protein (12, 17) or enzyme (19) profiles, and indirect immunofluorescence (15); none of these methods have been widely used. To date, plasmid content is the phenotypic characteristic most widely used to differentiate among strains of *Haemophilus ducreyi*. Several plasmids in *H. ducreyi* have been described; the most significant of these specify a Tem type β-lactamase which confers penicillin resistance (7, 10, 18).

We surveyed a collection of 30 isolates of *H. ducreyi* from Thailand for their plasmid content. Crude plasmid DNA preparations from cleared lysates (2) were examined by agarose gel electrophoresis, by using 0.75% agarose at 65 V for 3 h (9). Plasmid DNA was then transferred to nylon filters (Micron Separations, Inc., Westboro, Mass.) by the method of Southern (16). β-Lactamase-specifying plasmids were determined to be present if there was hybridization of the Southern blots of plasmid DNA with the radiolabeled *bla* gene isolated from the 4.4-MDa plasmid of *Neisseria gonorrhoeae* under stringent conditions (68°C, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–0.1% Denhardt's solution) (13). The hybridization results were confirmed by using crude plasmid DNA preparations to transform *Escherichia coli* DH5aMCR, as previously described (4). Penicillin-resistant transformants were detected on selective medium containing 200 μg of penicillin per ml. With the exception of a single isolate that possessed only a 5.7-MDa plasmid and allowing for the presence of open circular forms of plasmid DNA, each of the remaining 29 isolates possessed at least three different plasmids, as determined by the distribution of molecular masses observed (Table 1). Possibly eight different plasmids, ranging in size from about 1.8 to 10.0 MDa, were observed (Fig. 1). To determine whether any of the plasmids detected in these isolates were related to the β-lactamase-specifying plasmids in *H. ducreyi* described previously (7), eight isolates were examined more extensively (Fig. 1 and Table 2). The *bla* probe hybridized strongly with the 5.7- and 7.0-MDa plasmids and weakly with the 2.6-MDa plasmid. The 3.2-MDa plasmid present in these isolates did not hybridize with the *bla* probe, demon-

TABLE 1. Plasmid profiles of 30 isolates of *H. ducreyi* from Thailand

Plasmid content[a]	No. of isolates
1.8, 2.6, 2.8, 3.2, 5.7	3
2.8, 3.2, 4.4, 5.7	2
2.8, 3.2, 5.7, 7.0	2
1.8, 2.6, 2.8, 3.2	11
2.8, 3.2, 5.7	6
2.8, 3.2, 7.0	2
1.8, 2.8, 3.2	3
5.7	1

[a] Molecular masses of plasmids are given in megadaltons.

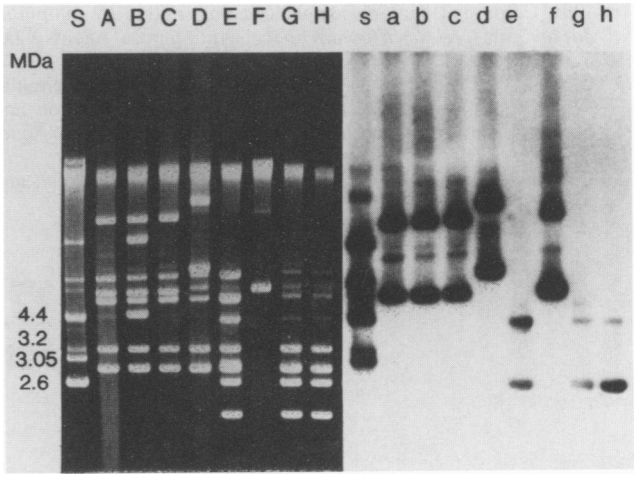

FIG. 1. Agarose gel electrophoresis of *H. ducreyi* plasmids. Lanes: A through H, plasmid DNA from isolates HD-216, HD-219, HD-227, HD-236, HD-243, HD-247, HD-307, and HD-312, respectively; S, 2.6-, 3.05-, 3.2-, and 4.4-MDa plasmids of *N. gonorrhoeae*; s through h, corresponding samples of the hybridized Southern transfer after autoradiography.

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TABLE 2. Hybridization of plasmids of *H. ducreyi* isolates with *bla* gene and transformation of *E. coli* DH5aMCR with plasmid DNA

Plasmid content[a]	No. of isolates	Hybridization[b]	Presence of transformants[c]
1.8, 2.6, 2.8, 3.2	3	±(2.6)	−
2.8, 3.2, 4.4, 5.7	1	+(5.7)	+
2.8, 3.2, 5.7	2	+(5.7)	+
2.8, 3.2, 7.0	1	+(7.0)	+
5.7	1	+(5.7)	+

[a] Molecular masses of the plasmids are given in megadaltons.
[b] Sizes of plasmids (in megadaltons) that hybridized with *bla* gene are given in parentheses.
[c] Penicillin-resistant transformants.

strating that it was unrelated to the 3.2-MDa β-lactamase-specifying plasmids in *H. ducreyi* and *N. gonorrhoeae* described previously (1). The 2.6-MDa plasmid that hybridized weakly with the *bla* probe may be identical to the 2.6-MDa plasmid previously described by McLean et al. (6). The presence of the β-lactamase-specifying plasmids was confirmed as described above; penicillin-resistant transformants of *E. coli* DH5aMCR were obtained only when the donor DNA contained either the 5.7- or the 7.0-MDa plasmid, but not the 2.6- or the 3.2-MDa plasmid (Table 2). In addition, the 2.8-MDa plasmid observed in the majority of these strains may be the kanamycin resistance plasmid described previously (14).

These isolates from Thailand appear to be unique; the number and diversity of the plasmids present in each of these isolates distinguish them from strains previously isolated in the United States, Canada, or Kenya (7). We have recently observed one isolate of *H. ducreyi* from a patient in San Francisco, Calif., however, that possessed a combination of the 1.8-, 2.6-, 2.8-, and 3.2-MDa plasmids. The extent to which strains from Thailand have contributed to the strain population found in the United States, Canada, and Kenya is unclear at present.

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