

Isolation of *H. ducreyi* from the male chancroid

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In the study of Thai penile ulcers, Out of the 8 clinically diagnosed Cases of chancroids, only one strain of H.ducreyi was finally confirmed by the Department of Microbiology, Jefferson Medical College of Philadelphia. U.S.A.

In this article, the clinical manifestation, the histo-pathological appearance of chancroid, the bacteriology of H.ducreyi, the substantial means of chancroid-diagnosis and also the etiology of chancroid are also discussed.

Also, this appears to be the first case report of identified H.ducreyi in Thailand.

นราทร ธรรมบุตร, ภิมย์ กมลรัตนกุล. การแยกวิเคราะห์จุลชีพ ฮีโมฟิลัส ดูครีฌี จากแผลริมอ่อนที่อวัยวะเพศชายไทย. *จุฬาลงกรณ์เวชสาร* 2528 สิงหาคม; 29 (10) :

คณะผู้วิจัย รายงานการแยกจุลชีพ จากแผลอวัยวะเพศของชายไทย ที่แพทย์ให้การวินิจฉัยทางคลินิกว่าเป็นแผลริมอ่อน จากผู้ป่วยแผลริมอ่อน 8 ราย, พบว่ามีเพียง 1 ราย ที่แยกวิเคราะห์ได้จุลชีพ แอโรบิก ฮีโมฟิลัส ดูครีฌี โดยมีการรับรองค้นพบจิมจากภาควิชาจุลชีววิทยา วิทยาลัย เจฟเฟอร์สัน, ฟิลาเดลเฟีย, อเมริกา นับว่าแยกเพาะเลี้ยงจุลชีพ ฮ. ดูครีฌี ได้เป็นครั้งแรกในประเทศไทย

อนึ่ง ในรายงานนี้ คณะผู้วิจัยยังรายงานลักษณะแผลริมอ่อน ลักษณะทางคลินิก จุลชีววิทยาของจุลชีพ ฮ.ดูครีฌี และวิจารณ์พยาธิกำเนิดของแผลริมอ่อน ว่า น่าจะเกิดจาก mixed infection มากกว่า เกิดจาก แอโรบิก ฮ.ดูครีฌี เพียงสายพันธุ์เดียว

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Human chancroid is an acute localized venereal disease probably caused by the short gram negative bacillus, *Hemophilus ducreyi*. Since other sexually transmitted diseases can mimic chancroid closely,⁽¹⁾ one should not make the diagnosis of Human H. ducreyi-chancroid on clinical basis alone. Beside clinical means, chancroid can be diagnosed by any of the following methods :

(1) recovery of *H. ducreyi* from the lesion and demonstrating it microscopically either by gram stain or fluorescein-tagged antibody.

(2) growing the organisms in vitro.

(3) intradermal reaction.

(4) autoinoculation. (Ito-Reenstierna skin test).

(5) biopsy⁽²⁾

(6) exclusion. (Unfortunately, many physicians make the diagnosis by exclusion.)⁽³⁾

In none of the clinically diagnosed chancroid has *H. ducreyi* been demonstrated as the etiologic agent. Indeed many clinicians make the diagnosis by the clinical characters of the initial penile ulcer alone.

The present report describe one case in which the organism was isolated from fresh specimens. This may be the first reported case in Thailand. Owing to the fact that the authors were on the research study* of "Bacteriology of Penile lesion" since January 1980* This paper presentation was then the advantage of the project.

A 30 year old, male Thai employee of The Si-Khew Jute Mill Company *

was seen at the VD-Clinic of Chulalongkorn Hospital,*** on the 4 th. June, 1981, due to a painful solitary ulcer located at the lateral side of his penile corona. He had the lesion for 4 days before visiting the Clinic and a history of sexual exposure without a condomization. After the intercourse he had the sensation of burning near the coronal sulcus. On the following day he noticed a minute lesion developing at the coronal sulcus of penis. He paid no attention to the lesion and believed that the ulcer.....would be "self-limiting" as it used to be. Neither cream, powder nor medication were administered. After four days interval, he came to the hospital with the larger, painful ulcer.

Physical examination revealed a 3 mm. diameter, localized ulceration on the lateral coronal sulcus. The base of the slightly lesion was glistening, reddish and shallow in appearance with rather clean necrotic tissue. The edge of the lesion showed signs of inflammation and the discharge was not foul-smelling. The clinical diagnosis was chancroid. Sampling technique : On the patient's first visit, 5 ml. blood was taken in a sterile tube for the V.D.R.L. test.****

He was privately interviewed with a series of questionnaires revealing that this was not his first V.D. exposure. He had the experience of several penile ulcers in different-penile locations. He had gonorrhoeae once in a while, and no history of underlying diseases.*****

1. In the case of chancroid and sus-

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** Si-Khew district of Nakorn Rajsima province, a north east city of Thailand.

*** Chulalongkorn Hospital OPD-Card No. 830052/81.

**** Venereal Diseases Research Laboratory Test.

***** Such as; cystitis, urinary tract infection, diabetes millitus and traumatic penile injury.

pected syphilitic lesion, special careful technique was used to obtain the specimens. Culture for *Hemophilus ducreyi*^(4,5) and dark field microscopy were immediately made from the material obtained from the base and margin of the ulcer with a sterile flattened platinum-wire. This material was inoculated into;

a) The serum overlaying freshly clotted human blood and incubated at 35° C in the ambient (5% CO₂), capneic (95% CO₂) and anaerobic environments.

b) The principal media used for isolation and cultivation was Mueller Hinton sheep blood agar. (BBL).

All plate-media were streaked for isolation, and a 5 ug. methicillin* and a 10 ug. ampicillin* disk were placed in area of heavy inoculation. The tubes and plates were examined daily for 7 days before discarded.^(4,5)

H. ducreyi was presumptively identified by Gram-stain, typical morphology and its tendency for specific arrangement. Presumptive positive isolates were finally confirmed at the Department of Microbiology, Jefferson Medical Centre, Thomas Jefferson University, Philadelphia, Pa, U.S.A.

2. The clinical specimen was carefully collected from the ulcer for bacteriological studies. Two sterile cotton-tipped swabs were used to obtain the clinical material from the ulcers. The first swab was inserted immediately into the original Stuart's medium and the modified Stuart's transporting medium and were promptly transported to the Laboratory.⁽⁶⁾ The second-

swab was used to prepare a smear on a clean slide, which was heat-fixed and Gram-stained.

Bacteriological processing : From the original and modified Stuart's media, the specimens were transferred to;

1. The Thayer Martin with Bacto-Hemoglobin, 1 per cent supplement B and the antibiotics selectivity. After 24-48 hours, 35° C in CO₂ atmosphere, the suspected colonies were investigated for *N.gonorrhoeae*.^(7,8,9)

2. The standard method of isolation and identification of aerobic organisms used in this study. Media for isolating aerobes and facultative anaerobes were Trypticase soy agar with.....5% sheep blood and 0.005% cysteine for the primary isolation, 1% Lactose brom-thymol blue agar and MacConkey's agar for Enterobacteriaceae or non fermentative Gram negative rods,⁽¹⁰⁾ APT agar (BBL)⁽¹¹⁾ for *Lactobacillus* sp., specific media for *Corynebacterium vaginale*,^(12,13) blood chocolate media for Neisseria group.

All staphylococcal infections were subjected to plasma coagulase test. The Gram-negative bacilli were traced by biochemical reactions.

Media for isolation of anaerobic bacteria were PRAS*** cooked meat glucose broth, fresh 5% sheep blood Trypticase soy agar, and specific media for *Bacteroides* spp., *Clostridial* spp.^(14,15) Anaerobic plates for strict anaerobes were duplicated studied in Anaerobic chamber, Anaerobic incubator, CO₂ Anaerobic cabinet for strict anaerobes,

* by using dark field condenser with phase contrast Spensor's American Optical Microscope No. 9068.

** Pure chemical disks from Bristol Company and not for therapeutic uses.

*** Pre Reduced Anaerobically Sterilized. Oxoid Company, England.

and Anaerobic jar with Gas generating kit (OXOID) for obligate anaerobes.

Identification of anaerobes were made on the basis of cellular morphology (Gram's stain) and colonial characters.^(14,15,16) Final identification was based upon the manual of the Virginia Polytechnic Institute of Anaerobes⁽¹⁸⁾

3. All media for anaerobic culture were incubated in the Anaerobic chamber as described by Narathorn.⁽¹⁷⁾

In our laboratory we possess the full scale anaerobiosis including Anaerobic incubator, Anaerobic CO₂ cabinet, Anaerobic chamber and Anaerobic jar with Gas generator kit. Other accessories are Gas-Liquid-Chromatography, and various biochemical tests according to the qualified procedures.^(18,19)

In case of definite diagnosis of chancroid, sulfisoxazole, (the drug of choice) were administered 4 grams per day for 1 week.^(20,21)

Local medication for general penile ulcer was Banocin powder* application after saline-soake and cleanliness were advised. Although the buboes usually subsided with the above therapy, the node should be aspirated in order to prevent spontaneous rupture.^(20,21)

After treatment, the patients were instructed to return for a final bacterial study, scheduled 10 days after the initial visit.

Laboratory investigations : Gram-

stained specimen showed many Polymorphonuclear leukocytes without intracellular organisms. Few lymphocytes were seen. Many gram positive cocci in pairs and clusters as well as the gram-negative coccobacilli, the gram negative fusiform bacilli were also seen. The red blood corpuscles, the fibrin pink-band and endothelial cells were scattered inbetween the organisms.

The blood VDRL was taken on the patient's first day of visit and later found to be "non reactive."

Bacteriology of *H. ducreyi*^(23,5,24)

Natural habitat : In smegma of normal male human being.⁽²³⁾

Gram's reaction : Intact microcolonies consisting of wavy rings of parallel chains of uniformly staining gram negative "School of fish" coccobacilli.

Growth requirement : Hemin and CO₂ requirement.⁽⁵⁾

Enrichment media :** Laboratories routinely using Mueller Hinton sheep blood agar (MHBC) and Mueller Hinton chocolate agar (MHCH).

Colonial character in MHBC; raised, opaque, compact, granular tan on yellowish pigment.

Specific biochemical properties : The only two biochemical characteristics are reduction of nitrate to nitrite and the production of alkaline-phosphatase.^(25,26,27)

B-lactamase production : They produce beta-lactamase ; resistance to the penicillin class antibiotics corresponded directly to this enzyme activity.⁽²⁸⁾

Rate of growth : Single colonies not visible until 48 hrs. of incubation.

* Each 10 grams contains Cloiquinol B.P.....0.3 gm., Bacitracin 750 IU., Neomycin sulfate 0.025 gm., Zine stearate 2 gm.

** with 1% Iso Vitale (BBL) in 150 mm. plate.

Table 1 Result of bacterial isolation and identification from the only one patient's chancroid

Characters of isolated colonies	Gram's reaction (arrangement)	Name of the isolated bacterial species.
1. Many round, smooth raised and glistening colonies.	Positive (in cluster)	Staphylococcus spp.
2. Few small round and central plateau with alpha hemolysis colonies.	Positive (pair and lancet shape)	<u>Streptococcus pneumoniae</u>
3. Few small round smooth pin point colonies in anaerobiosis.	Negative (pair and kidney shape)	Anaerobic Veillonella spp.
4. Few small round rough pin point colonies in anaerobiosis.	Negative (fusiform)	Anaerobic Fusobacterium spp.
5. Few raised, opaque, granular minute colonies with highly sticky consistency	Negative (school of fish pattern)	<u>H. ducreyi</u>
6. Few small round, smooth raised with irregular colonial edge-colonies in anaerobiosis.	Negative (pleomorphic form)	Anaerobic Bacteroides spp.
7. Few small raised opaque, glistening with regular colonial edge-colonies. Aerobic non hemolytic characters.	Positive (chinese arrangement)	Aerobic Diphtheroids.

* Difficult to pick up with a loop as colonies slide over agar surface; unable to emulsify into homogenous suspension.

Viability : Rapid deterioration. Parallel chains appear to coalesce. Typical morphology for only 1-2 days followed by amorphous debris. (ghost forms)^(25,26)

Maintenance : Continuous subcultures, as growth becomes visible, were necessary to maintain viability on artificial media. Preserves well in skim milk at -70°C at least 4 years.^(25,26)

Pathogenicity : Etiology of human chancroid, a sexual transmitted disease. No report on susceptible laboratory animal.

Susceptibility test : Resistance to all class of penicillin. Sensitive uniformly to cephalothin, gentamicin streptomycin and vancomycin. Of notable interest is the inverse

relationship in the responses, by three of the four beta-lactamase-negative strains to chloramphenicol and tetracycline in contrast to that seen to the penicillins. Of all the antibiotics tested, only erythromycin consistently shows large zones for all test strains.⁽²⁵⁾

Discussion

Of all the criteria in the diagnosis of chancroid, the clinical picture together with histopathologic appearances seems to be easier than by other means. (mentioned earlier)

At present, the *H. ducreyi* stain is claimed to be very helpful in microscopic morphology* in areas where laboratory

* Personal interview with Dr. Pairaj Desudjitr, Department of Preventive Medicines, Chulalongkorn Hospital Medical School, Bangkok, Thailand.

facility is limited. However, the reports of Leibovitz and Aoki have shown several organisms resembling *H. ducreyi*.^(29, 30) They also deteriorate rapidly.⁽²⁴⁾

The classical picture of Gram's stain of the carefully crushed suspected micro-colonies on a clean slide showing the specific characteristic of gram negative "School of fish" or "Finger print" bacilli is probably a promising one. However one must isolate the specific colonies on the specific media. In this report only one typical *H. ducreyi* is finally confirmed out of the 8 clinically diagnosed chancroids.** In view of this situation, *H. ducreyi* is probably not specific etiology of chancroid. As shown in Table 1, other bacterial flora, especially aerobic diphtheroids and anaerobic *Bacteroides* spp. are encountered. This mixed... infection may play a greater role than *H. ducreyi* in infected lesions^(22a, 22b) Moreover *Hemophilus ducreyi* has been cultivated from the smegma and vaginal secretions in patients without clinical manifestations of the disease. Such individuals may be carriers of the Ducrey bacillus⁽²³⁾ or the specific organism may be a normal flora.

Of all the 8 presumptive *Hemophilus* species, one of the *Hemophilus ducreyi* was finally mentioned by the Department of Microbiology, Jefferson Medical College. Therefore *H. ducreyi* is a doubtful causative agent of human chancroid.

From Table 1, aerobes as well as anaerobes were present in the penile ulcer as mixed infection. The aerobes (*Staphylococcus* spp., *Streptococcus pneumoniae*) may consume the oxygen in the lesion, and also release the "Growth factor" to their partners, the anaerobes (*Fusobacterium* spp., *Bacteroides* spp.). The mixed infection may then become the problem of "incurable" ulcer as mentioned by Gorbach.^(22b)

Moreover, Socransky and Gibbons have found the required role of *Bacteroides melaninogenicus* in mixed infections,⁽³¹⁾ therefore the isolation of aerobic diphtheroids and anaerobic *Bacteroides* spp. in the penile ulceration was rather significant. (Table 1) Both aerobes and anaerobes support not only Socransky and Gibbons' findings but also show the importance of mixed infections as the specific chancroid causative agents rather than the solitary specific *Hemophilus ducreyi*.

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