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Bacteriology of penile lesions

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นราทร ธรรมบุตร, กิรณย์ กมรัตน์กุล, กัญชลี เลิศโกยะสมบัติ,
สุศาลักษณ์ จันทรัชดา. จุลชีววิทยาของแผลที่อวัยวะเพศชาย. จุฬาลงกรณ์-
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คณะผู้วิจัยศึกษาแบคทีเรียในแผลอวัยวะเพศชายที่เกิดขึ้นเนื่องจาก เพศ
สัมพันธ์จำนวน 40 ราย และแผลที่มีได้เกิดเนื่องจากเพศสัมพันธ์ จำนวน 44 ราย

การศึกษาแสดงให้เห็นว่า

แบคทีเรียที่แยกได้จากแผลอวัยวะเพศ เป็นจุลินทรีย์หลายชนิด ทั้งแอโรบัสต์
และแอนแอโรบัสต์ร่วมกัน. จุลินทรีย์เหล่านี้มีความสำคัญและเป็นเหตุที่ทำให้แผลอวัยวะ
เพศหายช้า. (potential pathogens)

แบคทีเรียที่เชื่อกันว่าเป็นเหตุของโรคนั้น (specific pathogens) แม้ว่าจะ
แยกได้จากแผลอวัยวะเพศชายก็ตาม เมื่อให้การรักษาเต็มตามขนาดของยาที่กำหนด มี
หลายรายที่แผลไม่หาย.

การดูแลแผลอวัยวะเพศแผนใหม่ เป็นต้นว่า มิให้แผลสัมผัสน้ำและอื่น ๆ
(supportive treatment) ตามแนวที่คณะผู้วิจัยวางไว้ ร่วมกับการรักษาแบบ
มาตรฐาน (Conventional treatment) แล้ว ปรากฏว่าแผลอวัยวะเพศหายได้เร็วขึ้น.

ซิโมฟูลส คูรีนีย์ ซึ่งเป็นเหตุของโรคแผลริมอ่อนนั้น จากการศึกษานี้ แสดง
ให้เห็นว่า จุลินทรีย์นี้ น่าจะไม่ใช่ต้นเหตุของโรคแผลริมอ่อนที่แท้.

คณะผู้วิจัยยังวิจารณ์ความสำคัญของจุลินทรีย์ กลุ่มแอนแอโรบัสต์ (anaerobic
components) ใน mixed infections กับแผลที่อวัยวะเพศชายอีกด้วย.

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Penile lesions or ulcers are caused by many factors. The major ethiology of the ulcers are probably the traumatic injuries after sexual intercourse together with the infection from sexually transmitted diseases (S T D). The ulcerated penile fixed drugs reaction as well as other causative traumatic penile ulcers are also encountered as the minority. The bacterial isolation and identification of the male Thai's penile ulcers may reveal some advantages, namely;

1. Significance of data concerning the bacterial flora of the penile lesions.
2. The problem of delayed-ulcerative-healing after antibiotic administration.
3. The probable role of penile ulcerative-pathogenesis.
4. Is *Hemophilus ducreyi* definitely the cause human chancroid*?
5. Is the new idea of supportive treatment successful?

This research-work was studied at V.D.-Clinic, Department of Preventive Medicine, and Bacteriology-Division, Department of Medical Microbiology, Chulalongkorn Hospital Medical School, Faculty of Medicine, Chulalongkorn University, from January 9, 1980 through December 20, 1982.

The Subjects and Methods

A. The clinical subjects : The subjects consisted of 84 male patients who were examined at the Out-Patient-Department (V.D. Clinic) of Chulalongkorn Hospital with the symptoms of penile ulcers.

B. Sampling technique : On the patient's first visit, 5 ml. blood was taken in a sterile tube for the V D R L-test.** A series of questionnaires, consisting of age, primary or secondary V.D. exposure, spontaneous ulcerative lesion, characters and location of penile ulcer, history of underlying diseases (gonorrhoeae, cystitis, urinary tract infection, NIDDM*** or traumatic penile injury), was privately asked.

Those with the complete from of statements with the clinical diagnosis of penile ulcers were accepted in this study.

Patients were excluded if they were using topical or oral antimicrobials before-hand for the current problem.

The specimens were carefully collected from the ulcers of each patients 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

* **Chancroid :** is an acute localized-ragged ulcer on the genitalia with marked swelling and tenderness.

** Venereal Diseases Research Laboratory Test.

*** Non Insulin Dependent Diabetes mellitus.

were promptly transported to the laboratory.⁽¹⁾ The second swab used to prepare a smear on a clean slide, which was heat-fixed and Gram-stain.

In case of chancroid and syphilitic suspected lesion, special careful technique was used to obtain the clinical specimens for bacterial study.

C. The medication :^(2, 3)

C.1 The combination of Tetracycline* 2 grams/day for 1 week and Streptomycin 1 gm. intramuscularly for 5 days were given for all kinds of the penile ulcers except those who had the definite diagnosis of chancroid.

C.2 In case of definite diagnosis of chancroid, sulfisoxazole, were administered 4 grams per day for 1 week.

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, and the node should be aspirated in order to prevent spontaneous rupture.

After treatment, the patients were instructed to return for another additional visit. The second return for final bacterial study was 10 days after the initial visit.

D. Bacteriological processing : From the original and modified Stuart's media, the specimens were transported 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*.^(4,5,6)

2. The standard method of isolation and identification of the aerobic organisms was 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,⁽⁷⁾ APT agar (BBL)⁽⁸⁾ for *Lactobacillus* sp., specific media for *Corynebacterium Vaginale*.***^(9,10) blood chocolate media for Neisseria group.

All Staphylococcal infection were subjected to plasma coagulase test. The Gram-negative bacilli were traced to biochemical reactions.

* Those with the history of allergy to tetracyclines were switched to Ampicillin 2.5 grams/day for 1 week.

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

*** now called *Gardnerella vaginalis* or *Haemophilus vaginalis*.

Media for isolation of anaerobic bacteria were PRAS* cooked meat glucose broth, fresh 5% sheep's blood Trypticase soy agar, and specific media for *Bacteroides* sp., *Clostridial* sp.^(11, 12) Anaerobic plates for strictly anaerobes were duplicated studied within Anaerobic chamber, Anaerobic incubator, CO₂ Anaerobic cabinet for strictly anaerobes 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.⁽¹³⁾ Final identification was based upon the manual of the Virginia Polytechnic Institute of Anaerobes.⁽¹⁴⁾

3. All media for anaerobic culture were incubated in the Anaerobic chamber as described by Narathorn et al.⁽¹⁵⁾

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.^(14, 16)

4. Culture for *Hemophilus ducreyi*^(17, 18) and dark field examination*** were immediately made from material obtained from the *base and margin* of ulcer with a sterile flattened platinum-wire. This material was inoculated into;

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

4.2 The principal media used for isolation and cultivation were Mueller-Hinton Sheep blood agar. (BBL)

All plates were streaked for isolation, and a 5 µg. methicillin**** and a 10 µg. ampicillin**** disk in the area heavy inoculation. The tubes and plates were examined daily for 7 days before being discard.^(17, 18)

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.

* Pre Reduced Anaerobically Sterilized.

** Oxoid Company, England.

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

**** The pure-chemical-disks from Bristol Company not for therapeutic uses.

This project was studied mainly for Bacterial isolation.

The mycoplasma, the fungi, the virus or other causative agents were beyond the scope. The specimens for bacteriological analysis were processed in the Medical Microbiology, Bacteriology Laboratory (Chulalongkorn Hospital) immediately after collection.

The common isolated bacteria from the substantial penile ulcers were tabulated and compared in Tables 4-5. On the contrary, those who had returned for the re-check were incurable. The penile lesions were then re-study for the bacterial flora.

Our cases of penile lesion are mostly; (Table 3)

1. Chancroid.....	20-25 (37.5 %)	yrs. old.
2. Ulcers after oral sex.....	36-40 (37.5 %)	yrs. old.
3. Lesions after sexual exposure.....	20-25 (40 %)	yrs. old.
4. Syphilic like Chancres.....	26-30 (50 %)	yrs. old.
5. Spontaneous ulceration.....	26-30 (42.8 %)	yrs. old.
6. Ulcers after Herpes genitalis.....	41-45 (30 %)	yrs. old.
7. Ulcers after drug allergy.....	31-35 (50 %)	yrs. old.
8. Complicating ulcers.....	36-40 (31.2 %)	yrs. old.

However few of them returned for additional treatment (2 nd. visit) as shown in Table 1.

None of the patients had streptomycin sensitiveness or fixed tetracycline-reaction. Of all 84 patients, 40 cases were sexual exposure while 44 cases non sexual behavior.

In sexual ulcerative penile lesion, the highest bacterial isolation was *Staphylococcus aureus* (19.6 %) together with 19.61 % anaerobic *Peptostreptococcus* sp. (Table 4) both from the lesions after sexual exposure, while 17.02 % aerobic *Staphylococcus epidermidis* (spontaneous ulceration) and 36.36 % anaerobic *Peptostreptococcus* sp. (spontaneous ulceration) from those with non sexual exposure. (Table 5)

There were 10 cases with the NSU* as the underlying diseases. All of them had non-reactive VDRL and the Dark field illuminative microscopy were all negative,

As shown in Table 2, 44 cases were non sexual group and 40 cases with the history of sexual behavior. They haboured 45.23 % of symptomless, with highest coronal sulcus ulcerative location. (38.09 %) Most of the penile ulcer are solitary (69.04%) and the incubation period was more than 1 week. (40.47%) In our series, 67.85 % had no prepue-presentation.

* NSU : non specific urethritis.

Result

During Jan. 1980-Dec. 1982, the male patients with penile ulcerations were chosen for culture as shown in the Table 1.

Table 1 The patients' definite clinical penile ulcerative diagnosis

Definite diagnosis / Number of visiting patients	Patients' 1 st. visit	Patients' final visit	
Sexual exposure (40)			
1. Chancroids	8	3 (37.5%)	
2. Ulcers after oral sex	8	6 (75%)	
3. Lesions after sexual exposure	20	4 (20%)	
4. Syphilitic-like chancres (hard chancre)	4	1 (25%)	
Non sexual exposure (44)			
1. Spontaneous ulceration	14	4 (28.57%)	
2. Ulcers after Herpes genitalis	10	2 (20%)	
3. Ulcers after drug allergy	4	2 (50%)	
4. Complicating ulcers*	6	5 (31.25%)	
Total male patients	84	27 (32.14%)	

N.B :

* After circumcision, insect-bites, or traumatic injury.

Table 2 The result of questionnaires analysis

Questionnaires analysis	No. of Patients	%
1. History of :		
1.1 Sexual exposure, (40)*		
1.1.1 without condomization	28	28.70
1.1.2 with condomization	4	10
1.1.3 oral sexual exposure	8	20.0
1.2 Nonsexual relationships, (44)		
1.2.1 spontaneous ulceration	14	31.8
1.2.2 secondary ulcers after herpes viral infection	10	22.72
1.2.3 secondary ulcers after drug allergy**	4	9.09
1.2.4 complicating ulcers	16	36.36
2. Clinical manifestation (84)		
2.1 painful	10	11.90
2.2 pain (slightly)	18	21.42
2.3 fever (low grade)	12	14.28
2.4 fever and chill	1	1.19
2.5 headache	1	1.19
2.6 malaise	1	1.19
2.7 joint pain	6	7.14
2.8 foul smelling	14	16.66
2.9 inguinal lymph nodes involvement	8	9.52
2.10 symptomless	38	45.23
3. Location of penile ulceration, (84)		
3.1 glans penis	12	14.28
3.2 coronal sulcuts	32	38.09
3.3 shaft of penis	13	15.47
3.4 frenum location	14	16.66
3.5 skin of prepue	6	7.14
3.6 meatus location	4	4.76
4. Numbers of penile ulceratiyn, (84)		
4.1 single ulcer	58	69.04
4.2 double ulcers	20	23.8
4.3 multiple ulcers***	6	7.14

* () in parenthesis were the total cases in each group of questionnaires.

** Fixed drug eruption after antibiotic therapy for non ulcerative purpose.

*** more than 2 ulcers.

Table 2 (continued)

Questionnaires analysis	No. of patients	%
5. Incubation period, (84)		
5.1 less than one week	34	40.47
5.2 1 week	23	27.38
5.3 1-2 weeks	16	19.04
5.4 2-3 weeks	8	9.52
5.5 more than 3 weeks	3	3.57
6. Prepue-status, (84)		
6.1 present	21	25
6.2 absent	57	67.85
6.3 phymosis	6	7.14
7. Underlying diseases (84)	10	
7.1 Non specific urethritis	74	11.90
7.2 None		88.10

Table 3 Age distribution

Age ranges (years)	Chancroids	Ulcers after oral sex	Lesions after sexual exposure	Syphilitic like chancres (hard chancre)	Spontaneous ulceration	Ulcers after Herpes genitalis	Ulcers after drug allergy	Complicating ulcers
20 - 25	3 (37.5)*	0	8 (40)	1 (25)	0	1 (10)	0	2 (12.3)
26 - 30	2 (25)	2 (25)	4 (20)	2 (50)	6 (42.8)	2 (20)	1 (25)	1 (6.2)
31 - 35	2 (25)	1 (12.5)	2 (10)	1 (25)	2 (14.3)	1 (10)	2 (50)	2 (12.3)
36 - 40	1 (12.5)	3 (37.5)	3 (15)	0	3 (21.4)	2 (20)	0	5 (31.2)
41 - 45	0	2 (25)	2 (10)	0	1 (7.1)	3 (30)	1 (25)	1 (6.2)
46 - 50	0	0	1 (5)	0	2 (14.3)	0	0	2 (12.3)
51 - 55	0	0	0	0	0	1 (10)	0	2 (12.3)
56 - 60	0	0	0	0	0	0	0	1 (6.2)

* N.B.: In parenthesis were the percentage of the patients' penile lesions

**Table 4 The common isolated bacteria recovered
from the patients' penile ulcers
after sexual exposure (before and after conventional treatment)**

Bacterial genus (aerobes)	Before treatment		
	Chancroids (8)*	Ulcers after oral sex (8)	Lesions after sexual exposure (20)
<u>AEROBES AND FACULTATIVE</u>			
1. <u>Gram positive cocci</u>			
<u>Staphylococcus aureus</u>	6(9.37%)	6(12.5%)	10(19.60%)
<u>Staphylococcus epidermidis</u>	7(10.94%)	4(8.33%)	8(15.69%)
α hemolytic streptococci	2(3.12%)	2(4.17%)	2(3.92%)
β hemolytic streptococci	4(6.25%)	1(2.08%)	2(3.92%)
<u>Streptococcus fecalis</u>	6(9.37%)	2(4.17%)	0
<u>Streptococcus pneumoniae</u>	4(6.25%)	4(8.33%)	6(11.76%)
Non hemolytic streptococci	1(1.56%)	2(4.17%)	1(1.96%)
2. <u>Gram negative cocci</u>			
<u>Neisseriae gonorrhoeae</u>	1(1.56%)	0	4(7.84%)
Neisseria species	4(6.25%)	2(4.17%)	6(11.76%)
3. <u>Gram positive bacilli</u>			
Lactobacillus sp.	2(3.12%)	2(4.17%)	1(1.96%)
Diphtheroids	4(6.25%)	6(12.5%)	4(7.84%)
4. <u>Gram negative bacilli</u>			
<u>Escherichia coli</u>	4(6.25%)	1(2.08%)	1(1.96%)
<u>Enterobacter aerogenes</u>	2(3.12%)	3(6.25%)	2(3.92%)
<u>Klebsiella pneumoniae</u>	2(3.12%)	1(2.08%)	0
Proteus sp.	6(9.37%)	4(8.33%)	2(3.92%)
Pseudomonas sp.	4(6.25%)	3(6.25%)	1(1.96%)
<u>Hemophilus ducreyi</u> **	1(1.56%)	0	0
<u>Hemophilus vaginalis</u>	0	1(2.08%)	0
<u>Serratia macescens</u>	4(6.25%)	4(8.33%)	1(1.96%)
Total aerobic strains	64	48	51

* In parenthesis were the total selected cases of penile ulcer in each group.

** The isolated strain had been confirmed from the Microbiology Department of
Jefferson Medical Centre, Philadelphia, Pa, U.S.A.

	after treatment			
Syphilitic like chancres (hard chancres) (4)	Chancroids (3)	Ulcers after oral sex (6)	Lesions after sexual exposure (4)	Syphilitic like chancres (hard chancres) (1)
3 (12.5%)	3 (15.79)	4 (21.05%)	3 (21.42%)	1 (14.28%)
1 (4.17%)	3 (15.79%)	2 (10.53%)	1 (7.14%)	
1 (4.17%)	1 (5.26%)	1 (5.26%)	1 (7.14%)	1 (14.28%)
0	1 (5.26%)	1 (5.26%)	1 (7.14%)	0
0	1 (5.26%)	1 (5.26%)	0	0
2 (8.33%)	2 (10.53%)	1 (5.26%)	1 (7.14%)	1 (14.28%)
4 (16.66%)	0	0	0	1 (14.28%)
0	0	0	0	0
3 (12.5%)	1 (5.26%)	0	2 (14.3%)	1 (14.28%)
0	0	1 (5.26%)	0	0
3 (12.5%)	3 (15.79%)	3 (15.79%)	0	0
2 (8.33%)	2 (10.53%)	0	1 (7.14%)	1 (14.28%)
0	0	1 (5.26%)	1 (7.14%)	0
1 (4.17%)	0	1 (5.26%)	0	0
0	1 (5.26%)	1 (5.26%)	2 (14.3%)	0
2 (8.33%)	1 (5.26%)	1 (5.26%)	1 (7.14%)	0
0	0	0	0	0
1 (4.17%)	0	0	0	1 (14.28%)
1 (4.17%)	0	1 (5.26%)	0	0
24	19	19	14	7

Table 4 continued.

Bacterial genus (anaerobes)	Before treatment		
	Chancroids (8) *	Ulcers after oral sex (8)	Lesions after sexual exposure (20)
ANAEROBES			
1. <u>Gram positive cocci</u>			
Peptococcus sp.	2 (7.41%)	2 (4.65%)	6 (11.76%)
Peptostreptococcus sp.	5 (18.51%)	5 (11.63%)	10 (19.61%)
Anaerobic Gaffkya tetragena	1 (3.70%)	4 (9.30%)	2 (3.92%)
2. <u>Gram negative cocci</u>			
Veillonella sp.	2 (7.41%)	7 (16.28%)	6 (11.76%)
3. <u>Gram positive bacillis</u>			
Lactobacillus sp.	1 (3.70%)	2 (4.65%)	4 (7.84%)
Propionibacterium sp.	2 (7.41%)	3 (7%)	3 (5.9%)
Eubacterium sp.	0	0	0
<u>Clostridium perfringens</u>	1 (3.70%)	1 (2.32%)	2 (3.92%)
<u>Clostridium novyi</u>	1 (3.70%)	1 (2.32%)	0
<u>Clostridium sporogenes</u>	0	0	0
<u>Clostridium sordelli</u>	1 (3.70%)	0	0
4. <u>Gram negative bacilli</u>			
Bacteroides sp.	4 (14.81%)	6 (13.95%)	8 (15.69%)
<u>B.fragilis</u>	1 (3.7%)	2 (4.65%)	4 (7.84%)
<u>B.melaninogenicus</u>	3 (11.11%)	8 (18.60)	6 (11.76%)
Fusobacterium sp.	1 (3.7%)	2 (4.65%)	0
<u>F.nucleatum</u>	0	0	0
Unidentified	2 (7.41%)	0	0
Total anaerobic strains	27	43	51

* In parenthesis were the total selected cases in each group.

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บท 28 ฉบับที่ 7
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	after treatment			
Syphilitic like chancres(hard chancere) (4)	Chancroids (3)	Ulcers after oral sex (6)	Lesions after sexual exposure (4)	Syphilitic like chancere (hard chancere) (1)
0 1(10%) 1(10%)	1(8.33%) 0 1(8.33%)	0 1(6.67%) 1(6.66%)	1(7.69%) 1(7.69%) 0	0 1(33.3%) 1(33.3%)
1(10%)	0	0	2(15.38%)	0
1(10%) 0 0 0 0 0 0	0 1(8.33%) 0 1(8.33%) 0 0 0 0	0 2(13.33%) 0 0 0 0 0	1(7.69%) 1(7.69%) 0 0 0 0 1(7.69%)	0 0 0 0 0 0 0
3(30%) 1(10%) 2(20%) 0 0 0	2(16.67%) 2(16.67%) 3(25%) 1(8.33%) 0 0	4(26.67%) 0 4(26.67%) 0 0 0	2(15.38%) 2(15.38%) 2(15.38%) 0 0 0	1(33.3%) 0 0 0 0 0
10	12	15	13	3

**Table 5 The common isolated bacteria reeovered
from the patients' penile ulcers
of non sexual origin. (before and after conventional treatment)**

Bacterial genus (aerobes)	Before treatment		
	Spontaneous ulceration (14) *	Ulcers after Heres genitalis (10)	Ulcers after drug allergy(4)
<u>AEROBES AND FACULTATIVE</u>			
1. <u>Gram positive cocci</u>			
<i>Staphylococcus aureus</i>	2(4.25%)	6(15.38%)	1(5.26%)
<i>Staphylococcus epidermidis</i>	8(17.02%)	6(15.38%)	2(10.53%)
α hemolytic streptococci	6(12.76%)	4(10.26%)	1(5.26%)
β hemolytic streptococci	4(8.51%)	2(5.13%)	1(10.53%)
<i>Streptococcus fecalis</i>	0	1(2.56%)	0
<i>Streptococcus pneumoniae</i>	5(10.67%)	4(10.26%)	2(10.53%)
Non hemolytic streptococci	4(8.51%)	1(2.56%)	1(5.26%)
2. <u>Gram negative cocci</u>			
<i>Neisseriae gonorrhoeae</i>	0	0	0
Neisseriae species	4(8.51%)	2(5.13%)	0
3. <u>Gram positive bacilli</u>			
<i>Lactobacillus</i> sp.	1(2.13%)	0	1(5.26%)
Diptheroids	2(4.25%)	3(7.69%)	2(10.53%)
4. <u>Gram negative bacilli</u>			
<i>Escherichia coli</i>	1(2.13%)	4(10.26%)	1(5.26%)
<i>Enterobacter aerogenes</i>	1(2.13%)	2(5.13%)	3(15.78%)
<i>Klebseilla pneumonia</i>	2(4.25%)	0	0
<i>Proteus</i> sp.	3(6.38%)	1(2.56%)	2(10.53%)
<i>Pseudomonas</i> sp.	1(2.13%)	2(5.13%)	0
<i>Hemophilus ducreyi</i>	0	0	0
<i>Hemophilus vaginale</i>	0	0	0
<i>Serratia macescens</i>	3(6.38%)	1(2.56%)	1(5.26%)
Total aerobic strains	47	39	19

* In parenthesis were the total selected cases in each group.

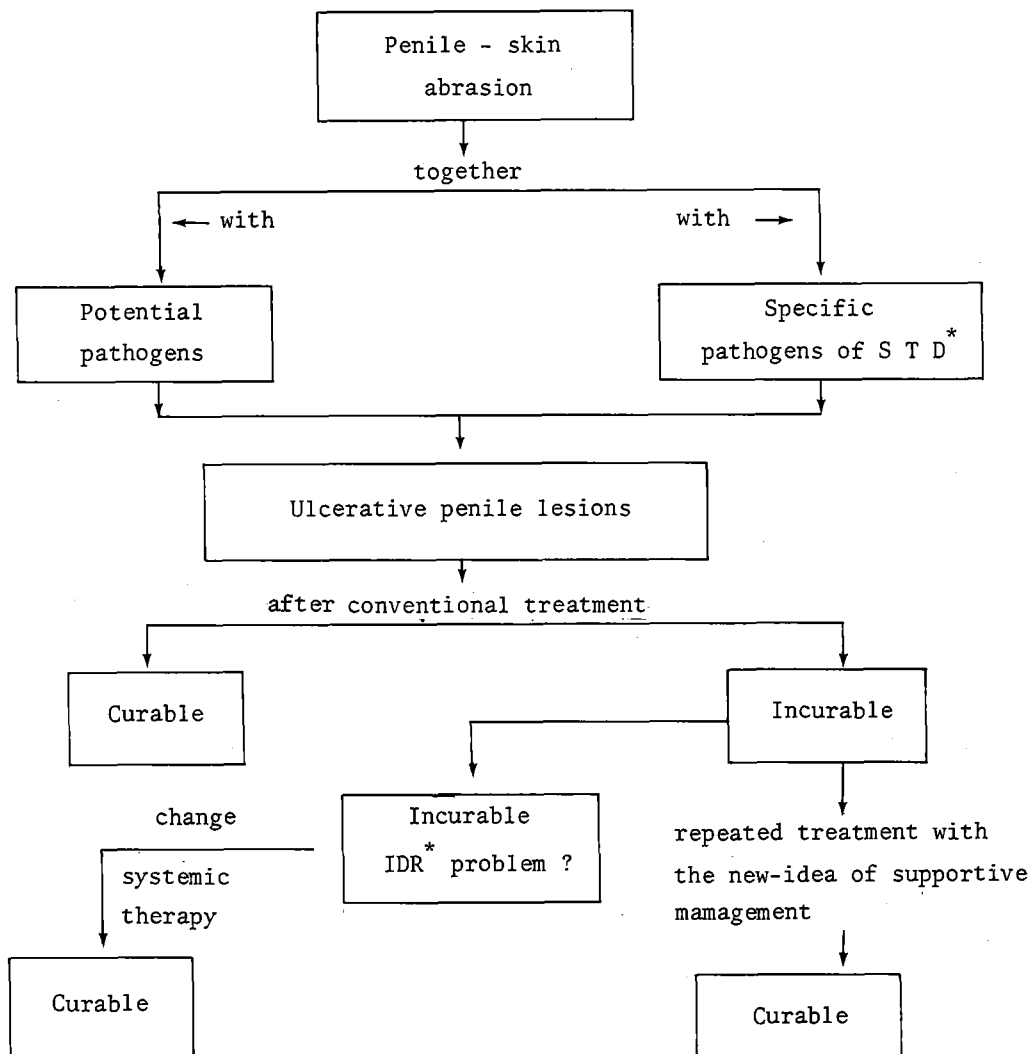
	After treatment			
Complicating ulcers(16)	Spontaneous ulceration(4)	Ulcers after Herpes genitalis (2)	Ulcers after drug allergy(2)	Complicating ulcers(5)
5(16.13%)	1(8.33%)	1(8.33%)	0	1(8.33%)
4(12.90%)	1(8.33%)	1(8.33%)	1(10%)	1(8.33%)
2(6.45%)	0	1(8.33%)	1(10%)	0
1(3.22%)	1(8.33%)	0	-0	1(8.33%)
2(6.45%)	0	0	0	1(8.33%)
2(6.45%)	1(8.33%)	2(16.67%)	1(10%)	2(16.67%)
1(3.22%)	1(8.33%)	0	0	0
0	0	0	0	0
4(12.9%)	1(8.33%)	1(8.33%)	1(10%)	0
1(3.22%)	1(8.33%)	1(8.33%)	0	0
2(6.45%)	0	2(16.67%)	1(10%)	1(8.33%)
1(3.22%)	2(16.67%)	1(8.33%)	1(10%)	2(16.67%)
3(9.7%)	1(8.33%)	0	0	1(8.33%)
0	0	0	0	0
0	1(8.33%)	1(8.33%)	1(10%)	0
1(3.22%)	0	0	1(10%)	1(8.33%)
0	0	0	0	0
0	0	0	1(10%)	0
2(6.45%)	1(8.33%)	1(8.33%)	1(10%)	1(8.33%)
31	12	12	10	12

Table 5 (continued)

Bacterial genus (aerobes)	Before treatment		
	Spontaneous ulceration (14)	Ulcers after Herpes genitalis (10)	Ulcers after drug allergy (4)
<u>ANAEROBES</u>			
1. <u>Gram positive cocci</u>			
Peptococcus sp.	5(22.73%)	1(11.11%)	1(10%)
Peptostreptococcus sp.	8(36.36%)	8(33.33%)	2(20%)
Anaerobic Gaffky tetragen 	1(4.54%)	0	0
2. <u>Gram negative cocci</u>			
Veillonella sp.	2(9.09%)	1(11.11%)	2(20%)
3. <u>Gram positive bacilli</u>			
Lactobacillus sp.	1(4.54%)	1(11.11%)	1(10%)
Propionibacterium sp.	2(9.09%)	0	0
Eubacterium sp.			
Clostridium perfringens	1(4.54%)	0	0
Clostridium novyi	0	0	0
Clostridium sporogenes	0	0	0
Clostridium sordelli	1(4.54%)	1(11.11%)	0
4. <u>Gram negative bacilli</u>			
Bacteroides sp.	0	1(11.11%)	2(20%)
B.fragilis	1(4.54%)	0	0
B.melaninogenicus	0	1(11.11%)	2(20%)
Fusobacterium sp.	0	0	0
Fusobacterium nuclatum	0	0	0
Total anaerobic strains	22	9	10

	After treatment			
Complicating ulcers(16)	Spontaneous ulceration(4)	Ulcers after Herpes genitalis (2)	Ulcers after drug allergy(2)	Complicating ulcers(5)
1(6.67%) 2(13.33%) 1(6.67%)	1(14.28%) 2(28.6%) 0	0 1(16.67%) 0	1(16.67%) 2(33.33%) 0	0 2(28.6%) 0
3(20%)	0	1(16.67%)	0	1(14.28%)
2(13.33%) 1(6.67%)	0 1(14.28%)	1(16.67%) 0	1(16.67%) 0	1(14.28%) 0
1(6.67%) 0 0 0	1(14.28%) 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
1(6.67%) 1(6.67%) 2(13.33%) 0 0	0 1(14.28%) 1(14.28%) 0 0	1(16.67%) 1(16.67%) 1(16.67%) 0 0	0 1(16.67%) 1(16.67%) 0 0	1(14.28%) 1(14.28%) 1(14.28%) 0 0
15	7	6	6	7

Diagram showing the probable role of
incurable-penile lesions



* STD = Sexual transmitted diseases

** IDR = Infectious drug resistance (22)

Diagram by the authors.

Discussion

As one may see, there are substantial characteristics of penile ulcers. The penile-skin abrasions are primary caused by both sexual and non sexual origins and followed by *the mixed flora*. In this situation, spontaneous lesions are really not spontaneous as mentioned earlier.

Imagine the size of the lesions, those with the diameters less than 1-2 mm. were practically impossible to take (scraping) the samples from the lesions. Therefore the specific ulcer should at least has the over 2 mm. diameter.

Formerly the classical concept of infection has been incorporated into the "Unitarian theory," One organism (such as pneumococcus) causes a specific disease (such as lobar pneumonia) and is treated by a single antimicrobial agent (such as penicillin). At present, mixed infections violate this rule since they are comprised of multiple organism with varying pathogenic potential. It might be useful to inquire why the mixed infection* seem to be favoured over monobacterial infection, There are several mechanisms that can be involved to explain this relationships among infecting microorganisms.^(19, 20)

Owing to our isolated bacteria of the ulcers, the organisms can be classified into 2 groups, namely;

a. The potential pathogens.

The isolated bacteria recovered from the lesions comprised the major part of the flora colonizing the skin and the female anogenital tract.⁽²¹⁾ These bacterial-flora, with some-modification, may then become mixed-infections to invade the skin abrasion and then turn to be pathogens.

b. The specific pathogens.

The real pathogens are also occasionally introduced into the lesion as shown in Table 4,5. The conventional therapy eradicates the specific pathogens, such as *Staph. aureus*, *N. gonorrhoeae*, *E. coli*, *B. fragilis*, *Peptostreptococcus sp.*, and *H. ducreyi*, therefore the penile ulcers should be cured completely. On the contrary, many penile ulcers were incurable and the bacterial flora (potential pathogens) are all recovered (final visit) as shown in Tables 1, 4 and 5.

Indeed, the potential pathogens are responsible for the incurable ulcers. Our findings demonstrate the significance of these bacterial agents in the ulcers and may probably be the problem of infectious drug resistance together with improper penile ulcerative management.⁽²²⁾

* poly-microbial infection.

In general, the conventional treatment of streptomycin and tetracycline for the penile-ulcers have high percentage successful cure-rate. However, there are some percentage of incurable. (Table 1) In our series, none of the cases is allergy to the specific treatment.

Questionnaires about clinical manifestation, a penile ulcer case may harbour many outstanding-symptoms, and joint pain is noted higher than others. (Table 2) The bacterial enzymes or toxins may be the cause of the specific clinical symptoms.

Those with the clinical symptoms of foul smelling have also the urethral discharge with the long prepues. (Table 2) This concludes that the combination of high percentage of mixed infections in the lesion and the bacterial end products of urethral discharges in the present of long prepue produce the dirty smelling. On the contrary, those with only penile lesions are non smelling. Therefore neither the urethral discharge nor the anaerobes in the lesion nor long prepue is probably the ethiology of bad-umpleasant odor.

About the biological screening test to exclude syphilis, although the sensitive simpler modification of the TPHA test* is developed, the standard VDRL for initial screening test is still the reliable one. To exclude syphilis ulcer the dark field microscopy is also suitable for the suspected penile ulceration.⁽²³⁾

The location of the penile lesion are meaningful. The frenum penile ulcers are painful and difficult to cure, because of permanant urine-contamination, especially those with long prepue. On the contrary, the meatus-penile ulcer is not difficult to heal owing to the drug-concentration in the urine. (Table 2)

About the age, penile ulcers-cases are seen both adolescent, adult with the history of predisposing agents. (Table 3)

From Table 4, our study emphasizes that all chancroids are not successfully cured by sulfisoxazole.^(2, 3)

Also from Table 4, out of 8 clinical diagnostic chancroid only 1 definite *H. ducreyi* is confirmed.** The fact that Lobovitz and Aoki^(19, 20) have been isolated "*H. ducreyi-like-micro-organism*" from the penile lesions, their works support our study that the specific chancroids are not always caused by *H. ducreyi*. Anaerobes and aerobes in the lesions may play the great role in chancroids and they can mimic *H. ducreyi* closely causing "Chancroid-like lesion."

About the *N. gonorrhoeae* isolation in the penile lesions, (Table 4) Chapel et al. have proved out that gonococci is the causative pathogens of the specific penile lesion.⁽²⁴⁾ We have no supportive evidence whether the isolated gonococci cause the penile ulcers.

* *Treponema pallidum* haemagglutination test.

** Final confirmatory from Dept. of Microbiology, Jefferson Medical College of Philadelphia, Pa, U.S.A.

This is really the matter of opportunist. (Table 4) However Narathorn has pointed out that Neisseriae species,* especially *N. catarrhalis** causes the specific penile lesion.⁽²⁵⁾

With the exception of oral sex penile lesion in Table 4, in fact they are probably not in the usual sexual form and therefore the isolated bacterial flora are then very much the same with those of non sexual group.

The pathogenesis of a penile ulcer can then be postulated as the primary skin abrasion followed by the potential pathogens or the specific pathogens according to the non sexual or sexual behaviors.

In view of our study, *Hemophilus ducreyi* is very difficult to isolate, and this organism is also the bacterial flora of smegma.⁽²⁶⁾ Furthermore after the specific chancroid treatment, few chancroid cases are seen incurable and one (later on) can not isolated *H. ducreyi*. polymicrobial flora is rather responsible for chancroid.

Those who had returned for the final visit (incurable), had been carefully suggested the new-supportive ulcerative treatment by neither soap, water, urine-contamination nor sweeping the ulcer by cotton nor tissue-paper. The authors' mentioned additional suggestion for local practical idea does harmless and no traumatic ulcerative tissue. In view of this situation with the second visit, the penile ulcers are dramatic curable by the same conventional treatment with our new idea of local ulcerative management. However in our study, only 84 penile ulcers are chosen for study and all of them are curable by the same standard of treatment.

The problem of infectious drug resistance may play the great role of trouble some if the cases are double.

Of special interests about the anaerobes from Table 4, 5 many anaerobes have been isolated, especially Bacteroides species. Our study then supports the polysaccharide capsule of *B. fragilis* for promoting ulcer-formation, also from table 4, 5, certain members of the infecting bacterial flora elaborate growth factors for each others.⁽²⁷⁾

The non pathogenic diphtheroids may release the essential factor (naphthaquinone closely related vitamin K.) for the potential pathogens.⁽²⁸⁾

Our observation reveals the bacterial flora of penile lesions are acquired and potentially turned to be pathogens.

Although all penile-cases were treated with the conventional drugs and those who returned for recheck (incurable ulcer) had no specific pathogens as

* The new name is *Branhamella catarrhalis*.

previously isolation, (Table 4, 5). In view of this situation, drug-susceptibility test was not required. However the trend of treatment should be improved so that the penile ulcers cases would be completely cured by the first visit only.

Moreover the possibility, for the delayed-penile-ulcer healing might be due to the isolated anaerobes. These species may relatively resistant to phagocytosis when compared to their aerobic counterparts, and anaerobes also interfere with Phagocytosis of aerobic organism.⁽²⁹⁾

This project expresses only the bacterial flora of the male penile ulcerative lesions. The female sexual-lesions are unfortunately beyond our scopes.

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