

Bacterial Flora of Health and Infected-Women's Vaginal and Cervical Areas*

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คลอดปากมดลูกในสตรี จุฬาลงกรณ์เวชสาร 2525 พฤศจิกายน; 26(6):529-541

คณะผู้วิจัยแยกวิเคราะห์จุลชีพแบคทีเรียในบริเวณช่องคลอด และปากมดลูกใน
สตรีไทย ณ หน่วยนรีเวช ภาควิชาสูติศาสตร์-นารีเวชวิทยา โรงพยาบาลจุฬาลงกรณ์
ตามเกณฑ์ที่ได้ตั้งไว้ตั้งแต่เดือนมิถุนายน 2523 ถึง มีนาคม 2524 ปรากฏว่า ในจำนวน
สตรี 258 ราย เป็นสตรีปกติ 96 ราย (ร้อยละ 37.27) สตรีที่มีโรคติดเชื้ออวัยวะสืบ
ช่องคลอดและปากมดลูก 112 ราย (ร้อยละ 43.4) ตัดทิ้งไปเพราะไม่เข้าเกณฑ์ 50 ราย

ผลศึกษาปรากฏว่า แยกได้จุลินทรีย์ทั้งแอโรบัส และแอนแอโรบัส 317
สายพันธุ์ โดยได้จากสตรีปกติ 134 สายพันธุ์ จากผู้ป่วยโรคติดเชื้อ 183 สายพันธุ์
จุลชีพจุลินทรีย์ที่แยกได้จากบริเวณช่องคลอด และปากมดลูก เมื่อนำร้อยละมาเปรียบ
เทียบ พบว่าไม่มีความสำคัญตามนัยแห่งสถิติ การศึกษาชี้ให้เห็นความสำคัญในการ
เก็บสปีชีเมนต์ให้ถูกวิธีการ, เพื่อได้แบคทีเรียที่ก่อโรคตัวจริง งานวิจัยนี้ ชี้ให้เห็นว่าโรค
ติดเชื้อในอวัยวะสืบสตรีนั้นเกิดขึ้นจากที่ช่องคลอดถูกรบกวนจนเสียดุลย์ปกติ การ
อักเสบจึงอาจเกิดจากจุลชีพประจำ ไมโครเบียล ฟลอรา หรือจากจุลชีพจรที่ถูกชักนำเข้า
มา ผลการวิจัยยังช่วยให้แพทย์ใช้เป็นหลักเบื้องต้นในการบริหารยาปฏิชีวนะในระหว่าง
รอผลจากห้องแล็บ โดยสามารถให้ยาต้านจุลชีพตามสายพันธุ์ที่แสดงอันเป็นเหตุสำคัญ
ส่วนใหญ่ที่ทำให้ติดเชื้ออย่างร้ายแรงด้วย อาจช่วยลดอัตราการตายได้

อนึ่ง การวิเคราะห์ทำให้ทราบว่าจุลชีพก่อโรคเป็นได้ทั้งแอโรบัส และแอน-
แอโรบัส

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Many Laboratories have studied the microflora of the female genital tract in health.^(1,2,3,) The major isolates have been aerobic and facultative bacteria; *strictly anaerobic bacterial are seldom isolated.* One possible explanation is that the previous studies of the normal flora and infected cervical flora did not use the anaerobic techniques for culturing the highly fastidious, oxygen sensitive bacteria. Moreover many recommended for anaerobic cultivation are too costly. The mentioned-situation causing the apparent lack of anaerobes in the normal flora is in contrast with their high incidence in pelvic infection.^(4,5) However 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.^(6,7) In view of this situation, we decide to analyse the prevalent organisms isolate from healthy and some gynecologic infections in order to solve;

1. The problem of true or false causative organisms.
2. The significance of the isolated vaginal and cervical microbial microorganisms.
3. The incidence of common isolated organisms for the primary management the women with severe gynecologic infections.
4. The important of isolated anaerobes and aerobes.

The subjects and Methods

a. Subjects. Two groups of women were studied.

The *first group* consisted of healthy women. They were asked to participate in the study if they were not pregnant, had not received antimicrobial agents during the preceding seven days, had no evidence of genital tract abnormalities, had not douched during the previous 24 hours, had regular menses and were not taking oral and parenteral contraceptives, had no in trauterine device. Reasons for attending the gynecologic clinic were contraceptive counseling, anual physical and cervical cytological examinations.

The *second group* of paticipants consisted of patients with gynecological complaints, and the clinical diagnosis were vaginal abscess, vaginitis, cervicitis, pelvic inflammatory diseases and septic abortions.

These individuals completed the questionnaires and the gynecologists also completed a form concerning observations the pelvic examination. The ages of these individuals ranged from 20 to 60 years.

b. Sampling technique. Specimens were collected with the use of paired sterile swabs from the subject's or the patient's vaginal and cervical areas. The first swab was inserted into the posterior vagina. Care was taken to avoid contact with the external genitalia and other sources of contamination. The second swab (same subject) obtained during the pelvic examination and was taken beyond

* Oxoid company, England.

the tip of the speculum. The paired swabs were immediately placed in the pre-reduced transport media in the butyl rubber-stoppered-glass bottle containing CO₂.

c. Bacteriological processing. The specimens for bacteriological analysis were processed in the Department of Medical Microbiology, Bacteriology Laboratory (Chulalongkorn Hospital) within 2 hrs. of collection.

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,⁽¹⁰⁾ APT agar (BBL)⁽⁸⁾ for *Lactobacillus* sp., specific media for *Corynebacterium vaginale*,^(9,11) blood chocolate media for Neisseria group.

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

Media for isolation anaerobic bacteria were PRAS* cooked meat glucose broth, fresh 5% sheep's blood Trypticase soy agar, and specific media for *Bacteroides* sp., *Clostridium* sp.^(12,13) 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.⁽¹⁴⁾ Final identification was based upon the manual of the Virginia Polytechnic Institute of Anaerobes.

From June, 1980 to March, 1981, 258 women in Gynecologic Clinic of Chulalongkorn Hospital were sampled for culture of both aerobic and anaerobic organisms. All subjects were classed as follow :

1. Normal healthy women 96 cases.
 - 1.1 Total paired specimens for culture 198 samples.
2. Infected genital tract infection.... 112 cases.
 - 2.1 Total paired specimens for culture 224 samples.
3. Unsatisfied subjects (gynecologic diseases, i.e. myoma uteri, pro-cidentia or incomplete questionnaires, etc.) 50 cases.
4. Total well and infected women 258 cases.

This project was studied mainly for bacterial-isolation and identification. The fungi, the parasites or other causative agents were beyond the scope.

* Pre reduced anaerobically sterilized.

Result

Of the total 258 subjects, 50 cases were unqualified while 96 normal healthy (37.2 per cent) women were chosen for study and 112 cases (43.4 per cent) were the infected gynecologic cases.

Out of 208 specimens from cervical areas, the total aerobic and anaerobic isolation were 317 strains (Table 3): 134 strains (42.3 per cent) from healthy and 183 (57.7 per cent) from the infected women. The specimens yielded polybacteria growth. The total isolated aerobes were 165 strains (52.0 per cent) and anaerobes were 152 strains (48 per cent).

In the healthy subjects the commonest facultative bacterium was the *Lactobacillus* sp. (24.7 per cent). There was no *Staphylococcus aureus* growth in the cervical area. The next common bacilli was *E. Coli*, other pathogenic and non pathogenic bacteria are shown in table 1. The anaerobes of the healthy subjects were *Bacteroides fragilis* (15.1 per cent) and *B. melaninogenicus* (18.9 per cent) in 53 subjects. The commonest anaerobes were Peptostreptococci in cases with clinical infection.

Of 112 gynecologic-patients examined, *Staphylococcus aureus* was highest identified (17.65 per cent) each in vaginitis from the cervical area. Other significant findings were *Lactobacillus* sp. (33.3 per cent) from vaginal abscess, *E. Coli* (40 per cent) from cervical area, *Proteus* sp. (20 per cent) from cervical

area, *Pseudomonas* sp. (8.33 per cent) from PID, *C. vaginale* (25 per cent) from cervicitis (Table 1), *Neisseriae* sp. (25 per cent) from cervicitis area (Table 1)

Peptostreptococci (80 per cent) from cervical area of vaginal abscess (Table 2), *C. perfringens* (20.5 per cent) from cervical area of PID (Table 2) *B. fragilis* (15.2 per cent) from vaginal area (Table 2), and *B. melaninogenicus* (6.5 per cent) from cervicitis (Table 2), Two strains of *Fusobacterium* sp. were seen.

The incidence of isolated organisms were tabulated and compared both items in Table 3. Mixed isolation of aerobes and anaerobes were high in infected women (41.7 per cent) while isolated-aerobes alone were high in normal healthy subjects. Only anaerobes were recovered at high percentage in cervicitis. However 14 (40 per cent) infected women and 14 (40 per cent) normal individuals were sterile (Table 3).

The amount of infected patients were 40 cases (35.7 per cent) of cervicitis, 30 cases (26.8 per cent) of PID,* 30 cases (26.8 per cent) of vaginitis, 8 cases (7.1 per cent) of vaginal abscess (3.6 per cent) of septic abortion (Table 3). The highest aerobic isolation was in vaginitis (42.7 per cent) and 36.1 per cent anaerobic identification in cervicitis (Table 3).

According to the age distribution, 29 (30.2 per cent) healthy subjects were highest between 26-30 years old, while 11 (27.5 per cent) of 26-30 years and 11 (27.5 per cent) of 31-35 years were

* PID = Pelvic inflammatory diseases.

Table 1 Comparison of the isolated aerobes between normal healthy and infected women.

Acrobies Isolated strains from;	Normal healthy subject (96)	Cervicitis (40)	Pelvic inflam- matory diseases (30)	Septic abortion (4)	Vaginal abscess (8)	Vaginitis (30)
	% *	%	%	%	%	%
<u>AEROBES AND FACULTATIVE</u>						
<u>1. Gram positive cocci</u>						
Staphylococcus aureus.....	-	-	-	2.5	11.1	17.6
Staphylococcus epidermidis.....	3.7	-	-	-	-	17.6
-hemolytic streptococci**.....	8.6	-	-	5.0	5.6	11.8
-hemolytic streptococci.....	3.7	-	-	2.5	-	-
Entero cocci.....	-	-	-	-	-	-
<i>Streptococcus pneumoniae</i>	2.5	-	-	2.5	-	-
Non hemolytic streptococci.....	7.4	-	-	10	5.6	5.9
<u>2. Gram negative cocci</u>						
<i>Neisseriae</i> sp.	4.9	25	20	5.0	5.6	-
<u>3. Gram positive bacilli. ...</u>						
<i>Lactobacillus</i> sp.	24.7	-	20	17.5	33.3	17.6
Diptheroids	4.9	-	-	5.0	5.6	5.9
<i>Corynbacterium vaginale</i>	3.7	25	-	2.5	22.2	5.9
<u>4. Gram negative bacilli</u>						
<i>Escherichia coli</i>	23.5	50	40	35	11.1	17.6
<i>Klebsella pneumoniae</i>	1.2	-	-	2.5	-	-
<i>Proteus</i> sp.	1.2	-	20	7.5	-	-
<i>Pseudomonas</i> sp.	2.5	-	-	2.5	-	-
Unidentified	7.4	-	-	-	-	-
Total strains	81	4	5	40	18	17
No growth specimens	2	0	1	0	4	0
Total specimens	96	40	30	4	8	30

* percentage of isolated strains.

** Including group A and non group A hemolytic streptococci.

Table 2 Comparison of the isolated anaerobes between normal health and infected women

Anaerobes	Isolated strains from;					
	Normal healthy Subject. (96)	Cervicitis (40)	Pelvic inflam matory diseases (30)	Septic abortion (4)	Vaginal abscess (8)	Vaginitis (30)
	%*	%*	%	%	%	%
ANAEROBES						
1. Gram positive cocci						
Peptococcus sp.	7.5	4.3	-	-	-	-
Peptostreptococcus sp.	15.1	43.5	41	50	80	50
2. Gram negative						
Veillonella sp.	7.5	4.5	2.6	25	-	-
3. Gram positive bacilli						
Lactobacillus sp.	-	-	5.1	-	-	-
Propionibacterium sp.	11.3	4.3	10.3	25	20	50
<i>Clostridium perfringens</i>	9.4	-	20.5	-	-	-
<i>Clostridium novyi</i>	-	13.0	-	-	-	-
4. Gram negative bacilli						
Bacteroides sp.	11.3	8.7	10.5	-	-	-
<i>B. fragilis</i>	15.1	15.2	7.7	-	-	-
<i>B. melaninogenicus</i>	18.9	6.5	2.6	-	-	-
Fusobacterium sp.	3.8	-	-	-	-	-
Unidentified	-	-	-	-	-	-
Total strains	53	46	39	4	5	4
No growth specimens	10	20	10	0	0	6
Total specimens	96	40	30	4	8	30

* percentage of isolated strains.

Table 3 Showing the number of women harbouring the organisms and the incidence of isolated organisms

Statements	No. of subjects or patients	No. of women harbouring the organisms				Incidence of isolated organisms (strains)	
		Mixed aerobes and anaerobes	Aerobes only	Anaerobes only	No growth	aerobes	anaerobes
Normal healthy subjects	96	40 (41.7%)	30 (31.2%)	12 (12.5%)	14 (14.6%)	81	53
Cervicitis	40 (35.7%)	6 (15%)	4 (10%)	22 (55%)	8 (20%)	4	46
Pelvic inflammatory diseases	30 (26.8%)	7 (23.3%)	3 (10%)	15 (50%)	5 (16.7%)	5	39
Septic abortion	4 (3.6%)	1 (25%)	1 (25%)	2 (50%)	-	40	5
Vaginal abscess	8 (7.1%)	1 (12.5%)	1 (12.5%)	6 (75%)	-	18	5
Vaginitis	30 (26.8%)	6 (20%)	10 (33.3%)	13 (43.3%)	1 (3.3%)	17	4
Total	208	61	49	70	28	165	152

Table 3 A. The χ^2 -test of the normal healthy's and infected women's isolated aerobes and anaerobes

Percentage of isolated strains from	Normal (96)	Cervicitis (40)	χ^2 - test	PID (30)	χ^2 - test	Septic abortion (4)	χ^2 - test	Vaginal abscess (8)	χ^2 - test	Vaginitis (30)	χ^2 - test
1. <u>Gram positive cocci</u> (%)			(34.9)		(30.8)		(15.21)		(15.11)		(0.05)
- aerobes and facultative	25.9	0		0		22.5		22.3		52.9	
- anaerobes	22.6	47.8	S	41.0	S	50.0	S	80.0	S	50.0	NS
2. <u>Gram negative cocci</u> (%)			(9.01)		(9.36)		(0.75)		(0.14)		(1.0)
- aerobes and facultative	4.9	25.0		20.0		5.0		5.6		0	
- anaerobes	7.5	4.3	S	2.6	S	25.0	NS	0	NS	0	NS
3. <u>Gram positive bacilli</u> (%)			(0.66)		(9.37)		(1.43)		(2.58)		(7.83)
- aerobes and facultative	33.3	25.0		20.0		25.0		61.1		29.4	
- anaerobes	20.7	17.3	NS	35.9	S	25.0	NS	20.0	NS	50.0	S
4. <u>Gram negative bacilli</u> (%)			(6.31)		(0.85)		(43.03)		(0.2)		(21.81)
- aerobes and facultative	35.8	50.0		60.0		47.5		11.1		17.6	
- anaerobes	48.2	50.4	S	21.6	NS	0	S	0	NS	0	S

N.B. : S = Significance P < 0.05 NS = non significance. P > 0.05

highest in number from cervicitis. Out of 30 PID, 10 (33.3 per cent) were between 26-30 years old, with 10 (33.3 per cent) of 26-30 years and 10 (33.3 per cent) were highest number of vaginitis (Table 4).

From Table 1.2 and 3, the most common isolated microorganisms from the infected women's cervical area were ;

1. *E. coli* and Peptostreptococci sp. (cervicitis)

2. *E. coli* and Peptostreptococci sp. (PID)

3. *E. coli* and Peptostreptococci sp. (septic abortion)

4. *Lactobacillus* sp. and Peptostreptococci sp (vaginal abscess)

5. *S. epidermidis* and Peptostreptococci sp. (vaginitis)

The statistic significance were also seen in Table 3A.

Table 4 Age-distribution of healthy and infected women

Ages (years)	Healthy women	Gynecologic infections.				
		Cervicitis	PID**	Septic abortion	Vaginal abscess	Vaginitis
20-25	16 (16.7%)	6 (15 %)	2 (6.7%)	-	-	5 (16.7%)
26-30	29 (30.2%)	11 (27.5%)	10 (33.3%)	2 (50%)	3 (37.5%)	10 (33.3%)
31-35	18 (18.7 %)	11 (27.5%)	8 (26.7%)	2 (50%)	-	10 (33.3%)
36-40	19 (19.8 %)	6 (15 %)	-	-	5 (62.5%)	2 (6.7%)
41-45	7 (7.3 %)	5 (12.5%)	4 (13.3%)	-	-	2 (6.7%)
46-50	3 (3.13%)	0	4 (13.3%)	0	0	1 (3.3%)
51-55	2 (2.08%)	0	2 (6.7%)	-	-	-
56-60	2 (2.08%)	1 (2.5%)	-	-	-	-
Total	96	40	30	4	8	30

* In parenthesis were the percentage of normal and infected women in each age group.

** PID = Pelvic inflammatory diseases.

Discussion

If the cervical area of the female genital tract is the central location, one may classify the gynecologic infections into 2 groups;

a. the infections around the cervical area the septic abortion, the pelvic inflammatory diseases and the cervicitis, etc.

b. the infections in the vaginal area are the vaginal abscess, the vaginitis, etc.

Originally, we had intended to obtain the data concerning the bacterial isolation between the vaginal and cervical areas of normal healthy and differential gynecologic conditions but lately found that the bacterial isolation of the two locations were almost the same amount and species.* Thus the dual samples were just for double check up.

However, several samples either from the normal or infected individuals revealed substantial per cent of "Growth". Many categories of this comparative can be drawn;

1. normal health subjects and the gynecologic patients regarding to the amount of isolated bacteria (Table 1,2). The aerobic Lactobacilli were higher in normal health while the amount were lower in the infected women, *C. vaginale* were higher than normal in the septic abortion, vaginal abscess. Only with the exception of *E. coli* were isolated higher in healthy than other infections.

The anaerobes such as Peptostreptococci sp., Bacteroides sp., *C. perfringens* were higher in the infections around the cervical area

2. number of normal healthy subjects and infected women harbouring the organisms (Table 3). The isolated *aerobes alone* as well as the isolated *aerobes with anaerobes* were seen highest in the normal healthy but the infected gynecologic case that harboured the combination or the single aerobic isolation were lower individually. The infection of pelvic inflammatory disease and the cervicitis cases were harboured by the higher amount of anaerobes.

The above 2 categories can be explained according to the fact that in pubertic period large amount of the specific carbohydrate (glycogen) present in the vagina. Lactobacilli attract the particularly carbohydrate and contribute for the maintenance of acidity (PH). There appears to be the disturbance mechanism in preventing the establishment of harmful microorganisms. In the infected women, the Lactobacilli incidence were lower in the vaginal vault (Table 1,2).

3. the normal healthy and infected gynecologic conditions on the amount of isolate aerobes and anaerobes.

In normal healthy the amount of aerobes in the vaginal canal were higher than the infected gynecologic cases (Table 3). Two possibilities concerned this problem, the alternative degree of

* No statistic significance.

oxygen-tension was the primary effected growth of aerobes and anaerobes. Secondly, the cervical mucous played the important role by possessing lysozyme, the anti-bacterial activity, which reduced the incidence of microorganisms in the cervical area. In the pre- puerperal infections the mentioned normal defense-mechanisms were disturbed, the percentage of the survival isolated microorganisms were probably increased in both areas.

Moreover, in our series, the total amount of aerobes number ed the anaerobes with the unknown explanation. This condition is different from the others.^(5,16)

4. the normal healthy and infected gynecologic diseases on the amount of "No growth."

Several samples either from the normal or infected individuals revealed substantial number of "No growth" because of technical errors and media preparation. Anaerobic-*no growth* were out number aerobes. (Table 1,2) Unfortunately very few anaerobic *Fusobacterium* sp. and also unidentified organisms were recovered. These isolated organisms were worth-nothing to mention about.

One can notice that there are 2 groups of isolated aerobes and anaerobes. The first group of isolated organisms was part of the microbial flora in the genito urinary tract (GU). The other minor group was the accidental bacteria, such as *S. aureus* or *S. epidermidis* However the common infections by these organisms have the potential of producing

the serious sequele of shock, disseminated intravascular coagulation, hemolysis, renal failure and death.^(15,16,17)

However this preliminary bacterial isolation offers the intersting points as follow.

1. In order to obtain the correct *causative organisms*, one should take the proper specimens from the area that relates to the clinical diagnosis and also knows how to collect the excellent samples for culture.

2. The disturbance of genital tract causes, firstly the high number of microbial flora, and these microorganisms are potentially pathogens. Secondly the accidental induced microorganisms are harmful.

3. The difference between microorganisms in genital tract in health and infected women seemed to be significance (Table 3 A 1-3).

4. The data about the most common bacterial isolation of gynecologic infections may be used as a presumptive indication for the inital treatment of choice until the specific bacteria are identified. Early antibiotic treatment based on the fixed antibiograms can prevent the serious complications.^(15,17)

Summary

258 women-subjects were chosen for study, but 50 subjects did not meet the study criteria and dropped out, etc. The total of 317 bacterial strains were identified with 134 strains (42.3 per cent) from healthy and 183 strains (57.7 per cent) from infected women.

One hundred and twelve common infected gynecologic patients of cervicitis (35.7 per cent), PID (26.8 per cent), septic-abortion (3.6 per cent), vaginal abscess (7.1 per cent) and vaginitis (26.8 per cent) were found to have 52 per cent positive bacterial culture of cervical and swab-samples.

Of ninety six well individuals, 134 strains (42.3 per cent) of aerobes and anaerobes were recovered from cervical area. The comparison of isolated bacteria revealed the significance relation-ship (Table 1-5). The project expressed the importance of specimens collection, the interference of cervical resident bacterial flora to become potentially pathogens,

and the useful data as the presumptive indication for the initial antimicrobial treatment.

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