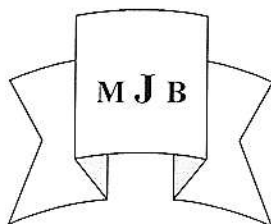


Isolation and Identification of *N. Gonorrhoeae* from Pregnant and Non Pregnant Women and Study the Response to Antibiotics

Ibtisam Habeeb Al-Azawi

Dent. of Microbiology, College of Medicine, Al-Qadissivah University



Abstract

During the period between January 2004 till September 2005, two hundred women were divided into two groups. One hundred pregnant and another non-pregnant in different ages beginning in 15 year to 45 year, were subjected to low vaginal swab in order to obtain specimens for determining the rate of vaginal colonization with *N. gonorrhoeae* and risk factor, which were obtained by direct questionnaire to obtain preliminary guide for the method of prophylaxis against many maternal and neonatal *N. gonorrhoeae* invasive diseases. Identification confirmatory test for *N. gonorrhoeae* has been done by using the conventional laboratory methods including gram stain, direct and indirect culture supplemented by other confirmatory biochemical methods (API₂₀ System, Niesseria). Twenty-three percent of the studied pregnant women were found to be colonized with *N. gonorrhoeae* as compared with non-pregnant control group in whom the rate of vaginal colonization was (18%). Some factors have been evaluated which may or not contribute to the increasing rate of colonization. Among these, age was found to be a significant risk factor in which the colonization rate was found to be high (68.2%) in women who are before 33 years of age. The parity were individually evaluated for their contribution as risk factors for the increase of *N. gonorrhoeae* colonization in which three and more were found to be associated with significant increase rate of *N. gonorrhoeae* colonization in addition to socioeconomic level. Regarding the antibiotics sensitivity, the results were significantly different from the known sensitivities reported in previous studies in which cefotaxime, cefazidime, ceftriaxon are still the most potent antibiotics. While the flouroquinole the lowest activity.

الخلاصة

خلال الفترة بين كانون الثاني 2004 وحتى شهر أيلول 2005، مائتا امرأة قُسمت على مجموعتين (مائة امرأة حامل والمائة الأخرى غير حوامل وفي أعمار مختلفة تبدأ من سن 15 سنة إلى 45 سنة) خضعن لمسحة مهبلية للحصول على عينة لغرض تحديد ودراسة نسبة تواجد المكورات السيلانية *N. gonorrhoeae* والعوامل المؤثرة لغرض الحصول على واسطة تقريبية لطرق الحماية من العديد من الأمراض التي تصيب الأم والطفل والتي تسببها البكتيريا المذكورة. ولغرض عزل البكتيريا المذكورة أعلاه استخدمت العديد من الفحوصات الاعتيادية (صبغة كرام، الزرع المباشر وغير المباشر) وعززت هذه الفحوصات بفحوصات أخرى كيميوجيوية (API₂₀ N. 20) حيث وجد أن (23%) من النساء الحوامل قيد الدراسة كن مستعمرات بالبكتيريا *N. gonorrhoeae* مقارنة بالنساء غير الحوامل حيث كانت نسبة الاستعمار (18%).

كما أن البعض من العوامل التي يمكن أن تؤثر على زيادة نسبة الاستعمار قد تم دراستها وتقييمها، إذ وجد أن نسبة الاستعمار تكون عالية (68.2%) عند النساء بأعمار 25-35 سنة، كما أن تعدد الإنجاب والمستوى المعاشي-الاجتماعي كانت من العوامل التي تم دراستها حيث وجد أن له تأثير على زيادة نسبة الاستعمار وكان المستوى المعاشي والاجتماعي الجيد يوفر حماية لنسبة من الاستعمار. وبالنسبة لحساسية البكتيريا للمضادات الحيوية ذات اختلاف ملحوظ عن النتائج السابقة، إذ ظهر أن السيفتالسم والسيفازديم والسفترينكون هي المضادات ذات الفعالية الأكثر في حين كان الفلوروكينولون هو الأقل فعالية.

Introduction

The epidemic of sexually transmitted diseases (STDs) are major global cause

of acute illness, infertility long-term disability and death, with severe medical and psychological consequences for

millions of men, women and infants, yet, STDs remains one of the most under recognized health threats in the country today. Despite the fact that STDs are extremely wide spread and have severe and sometimes deadly consequences, most people remain unaware of the scope of all but the most prominent STD. [1].

By far, women bear the greatest burden of STDs. The most serious STDs among women are chlamydia, gonorrhea and HIV. Both chlamydia and gonorrhea can lead to infertility, potentially fatal tubal pregnancies, and chronic pelvic pain. If not diagnosed and treated in time, during pregnancy, gonorrhea can result in serious health problems for infants. Moreover, women infected with chlamydia or gonorrhea are much more likely to be infected with HIV, if exposed. So, infection because of *N. gonorrhoeae* remains the second most common reportable disease in the United States [2].

In women, gonorrhea is a major cause of cervicitis and pelvic inflammatory disease. Pelvic inflammatory disease, in turn, can lead to ectopic pregnancy, infertility and chronic pelvic pain. Gonorrhea in pregnancy is associated with adverse outcomes including chorioamnionitis, premature rupture of membranes and preterm labor. Perinatal transmission to infants can cause severe conjunctivitis resulting in blindness if untreated and rarely sepsis with associated meningitis, endocarditis or arthritis [3].

Emerging evidence suggests gonococcal infection facilitates susceptibility and transmission of HIV in both men and women [4]. In 2003, the rate of reported gonorrhea in the United States was 116.2 cases per population of 100.000 with decreasing rates each year since 1999. This marks the lowest rate of gonorrhea ever reported by the CDCs. The prevalence of gonorrhea varies widely among regions of the country, with the

south reporting the highest rates (149.8 cases per 100.000 in 2003) [5].

Anti-microbial resistance in *N. gonorrhoeae* remains an important challenge to controlling gonorrhea. Gonococcal strains may be resistant to penicillins, tetracyclines, spectinomycin and fluoroquinolones. This resistance occurs as plasmid mediated resistance to penicillins and tetracyclines, and chromosomal-mediated resistance to spectinomycin and fluoroquinolones [6]. Because of these reasons, in addition to a lack of study about this subject in Al-Qadisiyah governorate, this study was conducted for isolation and identification *N. gonorrhoeae* from pregnant and non-pregnant women and to examine the anti-microbial activity of some antibiotics against it.

Materials and Methods

Sample Collection

A total of (200) samples were collected from women who attending to Maternity and Children Teaching Hospital / Department of Gynecology during the period from 2004 to 2005. These samples had been divided into two groups: pregnant (100), and non-pregnant (100), and were taken according to the method that was suggested by [6]. Inserting speculum, rub sterile cotton, swab against the posterior vaginal wall and allow swab to absorb specimen (a swab of the vaginal orifice can be taken if hymen is intact).

Identification

After the first step, the samples were carried to the laboratory of Medical College for culturing and bacteriological tests. All collected samples were cultured only aerobically by streaking on a selective media (e.g., Thayer Martin or Martin-Lewis) or non-selective media (e.g., Chocolate agar) if samples are from sterile or non-sterile respectively. Then inoculated media are incubated at 35°C-36°C in atmosphere supplemented with 5% CO₂ and examined at 24 hrs.

intervals for < 72 hrs. After that, bacterial isolates were purified and identified by depending on morphological characteristics which include shape of colony, edge of colony, fermentation of lactose sugar and nature of staining by gram's stain. In addition to biochemical tests by using API systems Nisseria [7].

Anti-microbial Agents

Anti-microbial agents were kindly provided by the following pharmaceutical companies, Cefotaxime (Rousel, La-defence-Interphar, Paris, France), Ceftriaxone (Roch Laboratories Nutleg, NJ, USA), Cefotazidime (Glaxo, Fortaz Research, Triangle Park, UK), Fluoroquinolone (Merck, Rahway, NJ, USA).

Determination of Susceptibility

By using disk diffusion method, which suggested by [7].

Data Processing and Statistical Analysis

The analytic statistical technique such as Chi-square test used for assessing the association of prevalence and the factor which is used [8].

Results

1. Prevalence of *N. gonorrhoea*:

Figure (1) demonstrates the incidence of vaginal colonization with *N. gonorrhoea* in the studied population pregnant women as compared with non-pregnant women as a control group. *N. gonorrhoea* colonization is significantly more frequent in pregnant women (23%) than control group (18%) (p value < 0.001).

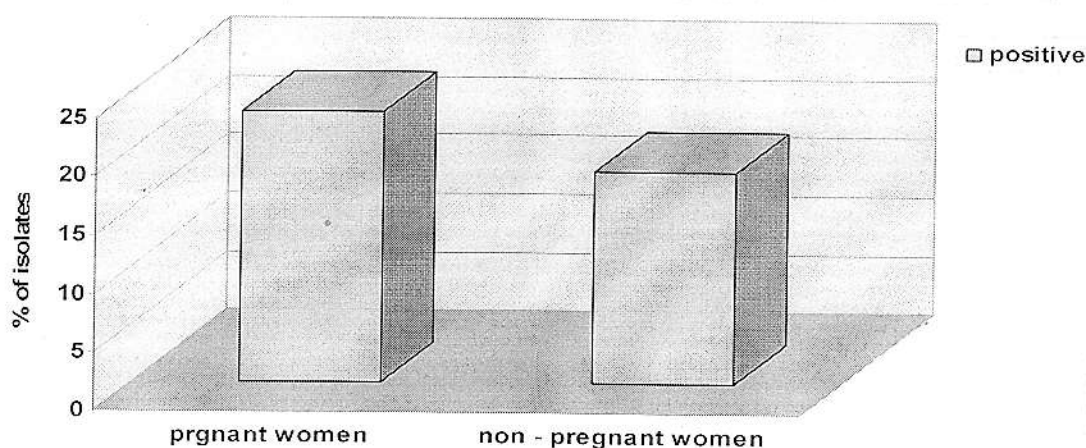


Figure 1 Rate of *N. gonorrhoea* in pregnant and Non - pregnant women

Age

The age group which mostly liable to have *N. gonorrhoea* in present study was 25-35 years which shows a rate of

(68.2%). The low rate was reported among women in the age 35-45 which was (14.6%) (Table 1).

Table 1 Frequency Distribution of *N. gonorrhoea* among Women by Age

Bacterial colonization	Age in Years					
	15-25		25-35		35-45	
	N	%	N	%	N	%
Positive	7	17.07	28	68.2	6	14.6
Negative	65	40.08	55	34.5	39	24.5
Total	71		83		46	200
$X^2 = 14.1$, d.f = 2, p < 0.001						

The Parity

Our study revealed that the highest frequency of *N. gonorrhoea* vaginosis occurs among women with

parity three and more. As (20), (48.7%) out of 200 women examined in this group affected by *N. gonorrhoea* (Table 2).

Table 2 Frequency Distribution of *N. gonorrhoea* among Women in Relation to Parity

Parity	Bacterial colonization				
	Positive		Negative		Total
	N	%	N	%	
One	10	24.3	54	22.9	64(32)
Two	11	26.8	75	47.1	86(43)
Three and more	20	48.7	30	18.8	50(25)
Total	41		159		200
$X^2 = 8.3, d.f = 2, p < 0.001$					

Socioeconomic Status

Patients with *N. gonorrhoea* vaginosis divided according to socioeconomic status into three groups: low, moderate, and high status. As

shown in table (3), we found that those patients with low economic status were present with higher rate (53.6%) of *N. gonorrhoea* vaginosis.

Table 3

Bacterial colonization	Socioeconomic status					
	Low		Moderate		High	
	N	%	N	%	N	%
Positive	22	53.6	10	24.3	9	21.9
Negative	44	27.6	49	30.8	66	41.5
Total	66		59		75	200
$X^2 = 13.2, d.f = 1, p < 0.001$						

Antibiotic Susceptibility

This test was done for (41) isolates from *N. gonorrhoea*, we found that most of *N. gonorrhoea* were susceptible to

cefotaxime (80.4%), ceftazidime (75.6%) and to lesser extent to ceftriaxone (65.8%). While the least one was to fluoroquinole (31.7%).

Table 4 Antimicrobial susceptibility of *N. gonorrhoea*

Antimicrobial agents	Cefotaxime	Ceftazidime	ceftriaxone	Fluoroquinole
Susceptible	33 (80.4%)	31 (75.6%)	27 (65.8%)	13 (31.7%)
Resistant	8 (19.5%)	10 (24.3%)	14 (34.2%)	28 (68.2%)
Total	41	41	41	41

Discussion

Attend maternal physiology during pregnancy places the mother at additional risk of certain infection. And subtle in their immunity may lead to

increase susceptibility to a variety of infections which result from disturbance in number and type of normal flora in the lining epithelia which permit the growth of new microorganism and after suitable circumstances to non-

pathogenic organism to be pathogenic [9].

The incidence of vaginal *N. gonorrhoea* colonization is variable in many countries. In this study, pregnant women were colonized by *N. gonorrhoea* by rate (23%) while in non-pregnant women was (18%), we can conclude that the pregnancy itself is an important risk factor for *N. gonorrhoea* colonization as compared with non-pregnant women, probably because of physiological changes in immune system and pregnancy related hormonal changes. In addition, the increasing size of the abdomen in third trimester of pregnancy may contribute to the difficulty in proper washing and cleaning of the vagina and the perineum [10].

The peak of *N. gonorrhoea* vaginosis in present study was in the age group 25-35 for which the higher colonization (68.2%) and lower incidence (14.6%) in women above 35 years. These results are consistent with other studies which reported an increasing incidence of *N. gonorrhoea* colonization in the youngest pregnant mothers [11]. The reason for this result is probably due to higher sexual activity.

Also, our study showed that the high rate of *N. gonorrhoea* colonization was (48.7%) which recognized with three and more parity, followed by (26.8%) among parity two. The lowest rate was (24.3%) which detected among women with unipara, table (2). This might be due to the fact that multiple pregnancy affected the design of behavior against symptoms of vaginal infection, or even against diseases in general [12].

In this work, the socioeconomic level of studied population has been classified into three groups (low, moderate, and high group). The highest incidence of vaginal *N. gonorrhoea* was found in women with lowest socioeconomic level (53.6%) which is

when compared with the high socioeconomic group (21.9%). It appears to be a risk factor for *N. gonorrhoea* colonization. This result can be explained by poor hygienic measures, nutrition and attendance of antenatal care and medical consultation for minor and irrelevant genital tract disorders was also poor in those with low socioeconomic level. Other studies have also demonstrated significant association between socioeconomic level and the incidence of *N. gonorrhoea* colonization [13,14].

Antibiotic sensitivity test is important for choosing the most appropriate antibiotic to which the bacteria is sensitive. Antibiotic sensitivity of *N. gonorrhoea* in present study, showed that most isolates (80.4%) were susceptible to cefotaxime, cefazidime (75.6%) and ceftriaxone (65.8%) while the majority of isolates (68.2%) were resistant to fluoroquinolone. These results were lower than that reported by previous studies which done by [15,16]. This finding means that there was a trend towards a more resistance against many antimicrobial agents, however, this difference may be attributed to the more empiric and prolonged antibiotics use toward which some *N. gonorrhoea* develop resistance strain.

Antimicrobial resistance in *N. gonorrhoea* occurs as plasmid mediated resistance to third generation cephalosporins and chromosomally mediated resistance to fluoroquinolones [14].

Finally, gonorrhea increases the risk of HIV infection (HIV; Human Immunodeficiency Virus, causes AIDS). So, prevention and early treatment of gonorrhea is critically important [2].

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