Evaluation of vaginal group B streptococcal culture results after digital vaginal examination and its pattern of antibiotic resistance in pregnant women

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Received: 3 March 2013
Accepted: 25 August 2013

Abstract

Background: Group B streptococcus (GBS) colonizes the gastrointestinal and genitourinary tract of 10-40% of pregnant women and it is a major cause of neonatal morbidity and mortality.

Objective: This study was to evaluate whether vaginal GBS culture results alter after digital vaginal examination or not. Antibiotic resistance pattern of this specie has been also assessed.

Materials and Methods: A total of 186 pregnant women with gestational age of 37 weeks were enrolled to the study. Two vaginal swaps were taken before and immediately after digital vaginal examination, then third swap was taken after 48 hours of examination. The cultures were evaluated for bacterial growth and the isolated bacteria were assessed for antimicrobial drugs sensitivity.

Results: Positive culture of GBS was seen in 16.1%. Initially negative GBS result was found not to change immediately after examination. But positive cultures were negative in 1.6% of women after digital vaginal examination. After 48 hours 2.7% of initially negative GBS was positive and no one with initially positive GBS had negative culture. Sensitivity to penicillin and vancomycin was 100%, erythromycin 74%, ampicillin 65%, cefazolin 62.8%, cefotaxime 54.2% and ceftriaxone was 40%.

Conclusion: The present investigation showed that the vaginal GBS culture result is minimally affected by digital vaginal examination. Drug of choice for GBS eradication is penicillin; vancomycin could be the choice in the case of penicillin hypersensitivity.

Key words: Vaginal examination, Streptococcus agalactiae, Pregnancy, Antibiotic.

This article extracted from M.D. thesis. (Mahdokht Sadat Manavi)

Introduction

Group B Streptococcus (GBS) are encapsulated gram-positive cocci which colonize 10-40% of pregnant women's gastrointestinal and genital tract (1). Although GBS colonization is usually asymptomatic in women, this is a known source of infection in neonates and infants. The most common cause of infection in newborns is GBS. Vertical transmission usually occurs at the beginning of labor or after rupture of membranes during delivery (2). This microorganism has been shown to be effective in adverse outcome during pregnancy including preterm labor, premature rupture of membranes, and chorioamnionitis, postpartum sepsis, pneumonia and meningitis. Also there are some reports of osteomyelitis and mastitis with GBS in the mothers after delivery (3). In the mid-80s, the researchers found that administration of penicillin and ampicillin during labor in GBS carriers can protect the infants from early infection (3).

On the basis of these findings, Center for Disease Control and Prevention (CDC) instructed to prevent streptococcal infections in infants at 2002. CDC says that pregnant women with recently positive vaginal GBS culture or those who have major risk factors for GBS colonization should take antibiotic prophylaxis. So this organization recommended screening and cultivation of all pregnant women with gestational age of 35 to 37 weeks (2). Digital vaginal examination (DVE) is a common examination which is performed on pregnant women and often

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collection of culture for group B streptococci is not considered until pelvic examination has been done using sterile gloves and lubricant gels. Dilution of the vaginal colonies after lubricant gel consumption may affect GBS culturing. There are controversial results reported previously by investigators about the effect of DVE on the vaginal culture (4).

This study was designed to evaluate the effect of DVE on the vaginal culture results and also find the sensitivity of GBS to different antibiotics in Arak city, Iran.

Materials and methods

Subjects
This was a cross-sectional conducted on 186 pregnant women with gestational age of 35-37 weeks. The subjects were recruited consequently from all pregnant women referred to the Taleghani hospital in Arak with convenience sampling, from September to November 2010. The study protocol was approved in ethics committee of Arak University of Medical Sciences and informed consent was obtained from participants before enrollment. Inclusion criteria were a pregnant women from 35-37 weeks who did not meet the exclusion criteria were included to the study. Exclusion criteria were history of DVE or intercourse within last 24 hours and suspicion of premature rupture of membrane (PROM).

Evaluation
The baseline characteristics and past medical and obstetric history of subjects were recorded. Patients were asked about their age, parity, gravity, medical history (hypertension, diabetes mellitus, epilepsy, and and pregnancy related diseases (preeclampsia, gestational diabetes mellitus, previous abortion, history of preterm labor, and anything else). The subjects were placed in lithotomy position for examination and sampling. Polyster swabs were taken from the lower vaginal sidewall before vaginal examination.

Then a vaginal examination was performed by physician using sterile gloves and lubricant gel, and after that vaginal swab was collected again. Third vaginal sample was taken 48 hours later. Speculum was not used. Swabs were inoculated directly onto blood agar (Quanda). Blood agar and EMB (Eosin Methylene Blue) were used for culture. For recognizing GBS, cAMP (Cyclic Adenosine Mono Phosphate) test and Ninhydrin were used. All suspected GBS colonies (beta-hemolytic, or non-hemolytic, Gram positive, catalase negative) were sub-cultured and isolated for confirmatory testing. Antibiogram was performed on blood agar using antibiotic disks including Penicillin, Vancomycin, Erythromycin, Ampicillin, Cefotaxime and Cefazolin.

Statistical analysis
The tendency indices are reported by mean and median and the dispersion indices are reported by standard deviation. The comparison of the culture results before and after DVE was done using McNemar test. Comparison between two independent groups was performed by Chi square and independent T-test. P value less than 0.05 was considered significant. All analyses were performed using SPSS V.16.

Results
The participants were 186 pregnant women with gestational age between 35 to 37 weeks and the mean age of 25.9±6.08 years (16 to 39 years old). From all subjects 15 (8%) had underlying diseases (hypertension, epilepsy and etc) and 13 (7%) had gestational diabetes mellitus and 8 (4.3%) had pregnancy induced hypertension. The baseline characteristics could be seen in table I. The results of culture before, immediately and 48 hours after vaginal examination were used in table I.

There was no significant difference between vaginal GBS culture before and after DVE (p=0.25). The vaginal culture was positive for GBS in 30 cases (16.1%) before DVE. After digital examination, from the 30 initially positive GBS subjects 27 (90%) were positive and the left 3 (10%) had negative culture result. None of the initially negative subjects had positive GBS results immediately after DVE (Table II). After 48 hours of first sampling and digital vaginal examination, 5 (2.7%) initially negative women were positive for vaginal GBS and the other 151 (81.2%) were negative. All previously positive subjects
(n=30, 100%) had positive culture of GBS after 48 hours. This difference was not statistically significant but it was borderline (p=0.063) (Table II).

There was no smoker among negative GBS subjects but 31 (19.9%) were passive smoker. From 30 positive subjects 2 persons (6.7%) were smoker and 1 (3.3%) was passive smoker. This difference was statistically significant (0.001). From 156 negative GBS participants 1 (0.6%) had consumed antimicrobial therapy in the last 7 days, 25 (16%) within 8-60 days before and 21 (13.5%) from 61-365 days before the study. Of the 30 women who had positive cultures of streptococci 1 subject (3.3%) during the last week, 2 (6.7%) during 8-60 days and 6 subjects (20%) between 61-365 days before the examination was taking antibiotics (p=0.26).

The association of the baseline characteristics and clinical condition with culture results could be seen in table I.

**Antibiogram**

The sensitivity of vaginal GBS was 100% to Penicilline and Vancomycin, 76% to Erythromycin, 65% to Ampicilline, 62.8% to Cefazolin, 54.2% to Cefotaxime and 40% to Ceftizoxime (Table III).

### Table I. Baseline and difference of baseline characteristics and clinical condition subjects characteristics and clinical condition of between subjects with positive or negative GBS before vaginal examination

| Variable                          | Measurement | Negative culture | Positive culture | p-value |
|-----------------------------------|-------------|------------------|------------------|---------|
| Age (years) (mean±SD)             |             | 25.9±0.4         | 25.76±6.39       | 26.83±4.02 | 0.21**  |
| Smoking [number (%)]              |             |                  |                  |         | 0.001***|
| Non smoker                        | 152 (81.7%) | 125 (80.1%)      |                  |         |
| smoker                            | 2 (1.1%)    | 0                |                  |         |
| Passive smoker                    | 32 (17.2%)  | 31 (19.9%)       |                  |         |
| Use of antibiotics [number [%]]   |             |                  |                  |         | 0.26*** |
| Past 7 days*                      | 2 (1.1%)    | 1 (0.6%)         |                  |         |
| Past 8-60 days*                   | 27 (14.5%)  | 25 (16%)         |                  |         |
| Past 61-365 days*                 | 27 (14.5%)  | 21 (13.5%)       |                  |         |
| No                                | 130 (69.9%) | 109 (69.9%)      |                  |         |
| Underling disease [number [%]]    |             |                  |                  |         | 0.092***|
| Chronic HTN                       | 1 (0.5%)    | 1 (0.6%)         |                  |         |
| Seizure                           | 1 (0.5%)    | 0                |                  |         |
| Diabetes mellitus                 | 3 (1.6%)    | 3 (1.9%)         |                  |         |
| Cardiovascular                    | 10 (5.4%)   | 3 (1.9%)         |                  |         |
| None                              | 171 (91.9%) | 171 (91.9%)      |                  |         |
| Obstetrics and pregnancy related diseases [number [%]] | | | | 0.19*** |
| GDM                               | 13 (7.0%)   | 13 (8.3%)        |                  |         |
| GHTN                              | 8 (4.3%)    | 8 (5.1%)         |                  |         |
| Abortion                          | 18 (9.7%)   | 12 (7.7%)        |                  |         |
| Preterm labor                     | 1 (0.5%)    | 0                |                  |         |
| None                              | 144 (77.4%) | 144 (77.4%)      |                  |         |
| Parity                            |             |                  |                  |         | 0.41*** |
| Nuliparous                        | 109 (59%)   | 92 (58%)         |                  |         |
| Multiparous                       | 77 (41%)    | 64 (41%)         |                  |         |
| STD [number %]                    |             | 5 (2.7%)         | 3 (1.9%)         | 2 (6.7%) 0.14*** |
| UTI during pregnancy [number %]   |             | 24 (12.9%)       | 19 (12.2%)       | 4 (13.3%) 0.89*** |

*days before the study
**independent student’s t test
***chi square
STD: sexual transmitted diseases.
UTI: urinary tract infection.
GDM: gestational diabetes mellitus;
GHTN, gestational hypertension.

### Table II. Streptococcus culture before and after digital examination and after 48 hours

| Variable                          | Result of culture before DVE | p-value |
|-----------------------------------|------------------------------|---------|
| Culture after DVE                 | Negatives | Positive |         |
| Negative                          | 156 (83.9%) | 3 (1.6%)   | 0.25*   |
| Positive                          | -         | 27 (14.5%) |         |
| Culture 48 hour after DVE         | Negatives | Positive |         |
| Negative                          | 151 (81.2%) | -         | 0.063*  |
| Positive                          | 5 (2.7%)  | 30 (16.1%) |         |

*chi square
DVE: digital vaginal examination.
Table III. Antibiotic sensitivity of isolated vaginal GBS in positive cultures

| Antibiotic      | Sensitivity (%) | Resistance (%) |
|-----------------|-----------------|----------------|
| Penicillin      | 100%            | 0%             |
| Vancomycin      | 100%            | 0%             |
| Erythromycin    | 76%             | 24%            |
| Ampicillin      | 65%             | 35%            |
| Cefazolin       | 62.8%           | 37.2%          |
| Cefotaxim       | 54.2%           | 45.8%          |
| Ceftizoxime     | 40%             | 60%            |

Discussion

In four recently performed studies on Iranian pregnant women with the gestational age of 35-37 weeks the vaginal streptococcus group B was reported 10-20% (1, 5-7). The GBS has been reported positive in Oklahoma in 19.5%, New York in 11.9%, Texas in 12.8% and Brazil in 17.9% of studied pregnant women (8-11). Based on the results of the present study the incidence of GBS in pregnant women of Arak is in the range of other cities and countries. The differences could be resulted from different racial, geographic, socioeconomic status, different sampling and organism identifying methods.

Considering the results of the present study digital vaginal examination caused 1.6% false negative and no false positive GBS infection. In a study by Knudston et al on 302 pregnant women, vaginal GBS culture after DVE was negative in 28.83% of initially positive women and it was positive in 5.3% of initially negative subjects. This difference was not significant but they suggested performing sampling before DVE (8). This difference may be due to difference in examiner, amount of lubricant that used or how the examination is done. Using a lot of lubricant may lead to false negative results. Same results were seen by Schwope and colleagues and they suggested performing sampling before DVE (9).

But Brady and colleagues reported that DVE using lubricant gel did not change the results of GBS culturing of 50 pregnant women and all post examination culture results was thesame as initial results (4). In the third sampling after 48 hours 2.7% of previously negative cases showed positive results and none of the initially positive women had negative culture. This may be due to transferring of the epidermal microbial into the vagina despite sterile setting. No association was found between gestational diabetes mellitus and the results of GBS cultures in the present study which was in line with the some previous studies (12, 13).

But in a study at 2010 vaginal streptococcus culture results was in relation with gestational diabetes mellitus in 201 pregnant women (14). Matorras and colleagues reported more group B streptococci in diabetic pregnant women (15). These different results may be due to the samples; both gestational and chronic diabetes mellitus were included as one group into the analysis previously but we only evaluated gestational diabetes mellitus. The results showed more abortion history in the women with positive GBS. The previous abortion may be due to the group B streptococci colonization in the vagina wall of these women (16).

Significant relation was seen between cigarette smoke exposures (passively or actively) and GBS cultures, higher smoke exposure was associated with higher rate of vaginal GBS colonization. These findings confirmed the results of Terry and colleagues reporting that 33% of smokers and 16.4% of nonsmokers had positive strep culture and smoking was suggested as a risk factor for maternal streptococcal colonization (17). In contrast to these findings Stapleton et al showed that non-smoker women had more positive GBS culture of their vagina and Zusman et al did not report significant association between smoking and vaginal streptococcus in 598 pregnant women (13, 11).

Urinary tract infection (UTI) did not have association with vaginal GBS in the present study that may be due to the previous antibiotic therapy of UTI in pregnant women.
Antibiotic consumption could lead to less microorganism colonization in the vagina. Vaginal Streptococcus did not have association with parity herein which was in line with the findings of Stapleton et al (13). But Regan and colleagues reported less GBS in multipar women (18). The sensitivity of vaginal GBS was 100% to penicillin and vancomycin, 76% to erythromycin, 65% to ampicillin, 62.8% to cefazolin, 54.2% to cefotaxime and 40% to ceftizoxime in the present study in Arak. According to an evaluation involved four states in the USA, group B streptococci was 100% sensitive to penicillin, ampicillin, cefazolin, cefotaxime and vancomycin, 87.3% to clindamycin and 74.6% to erythromycin (19).

In Ahvaz sensitivity to penicillin was 56%, ampicillin was 50%, cefazolin was 97% and cefotaxime was 88% (1). In Texas none were resistant to ampicillin but erythromycin resistance was 9%, 13% was resistant to clindamycin and resistance to cefazolin was 4% (20). There are many other studies evaluating the antibiotic resistance of GBS within different population of USA, Kuwait and Ardabil (Iran) (6, 21). According to the results of the different studies, in most areas GBS are 100% sensitive to the penicillin and vancomycin which is confirmed by the present study. But resistance to ampicillin in Arak (35%) was higher than other areas. There is also high resistance to cefazolin (37.2%) in the present study in Arak which is significantly higher than other areas.

Acknowledgments

This study was founded by Arak University of Medical Sciences. We want to appreciate the president of Taleghani hospital, Dr. Nourbakhsh, and technicians of laboratory of that hospital and also we would like to express our sincere gratitude to Farzan Institute for Research and Technology for technical assistance.

Conflict of interest

There is no conflict of interest in this paper.

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