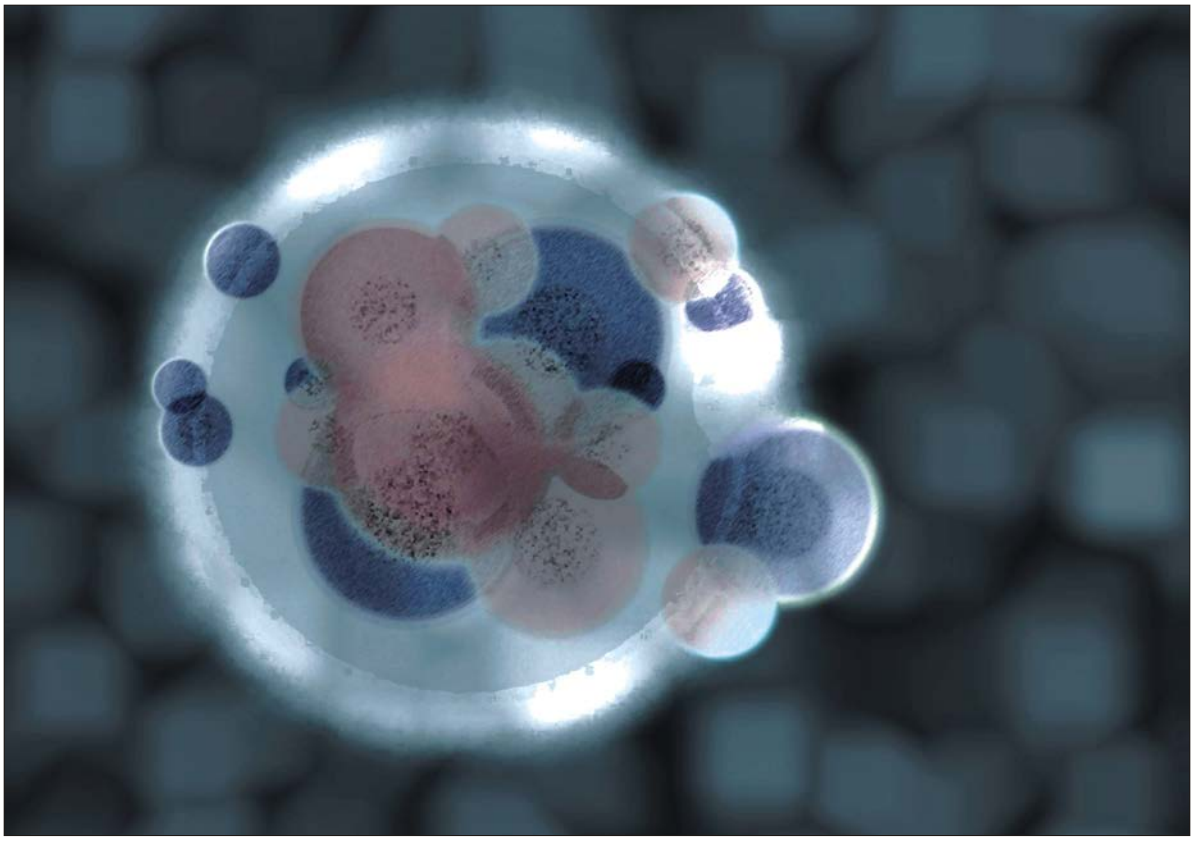

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Recto-Vaginal Colonization of Group B Streptococcus in Pregnant Women Referred to a Hospital in Iran and its Effect on Lactobacillus Normal Flora

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Abstract: Maternal recto-vaginal colonization of group B streptococcus in pregnant women visiting a hospital in Mashhad-Iran was studied. Recto-vaginal samples obtained from 201 pregnant women were carefully observed by direct Gram stain. Also, the samples were inoculated to Todd-Hewitt broth containing supplements. After re-inoculation onto sheep blood agar, group B streptococci were identified by colony morphology, beta-hemolysis, differential biochemical tests and agglutination test as confirmation identification. 25 (12.44%) of 201 pregnant women were GBS carriers; 24 (11.94%) and 22 (10.95%) positive for rectal and vaginal swabs, respectively. There were 2 (28.57%) carriers among 7 diabetic studied pregnant women. Gram positive bacilli suspected to lactobacilli in direct Gram stain of GBS carriers were observed less than non carriers, therefore replacing vaginal normal flora using lactobacilli as probiotic is recommended to prevent GBS infections in mothers and their neonates.

Key words: Group B streptococcus, recto-vaginal, colonization, carrier, pregnant women

INTRODUCTION

The Group B Streptococcus (GBS) is a common resident of the pregnant women's genital and intestinal tract (Franciosi *et al*., 1973; Badri *et al*., 1977; Matorras *et al*., 1989; Heath and Anne, 2007). Early-onset infections with this orgamism appear within 7 days after birth and contain about 80% of group B streptococcal infections in infants. Early-onset infections are acquired by contact with the genital tract of the mother during passing through the birth canal (Matorras *et al*., 1989; Bergeron *et al*., 2000) or by the ascending transition of this organism in uterus via ruptured membranes (Matorras *et al*., 1989). Approximately 60% of infants born become colonized with their mother's organisms (Patrick *et al*., 2002). Prevalence of colonization with group B streptococci among pregnant women was reported from 4 to 40% (Matorras *et al*., 1988).

Some complications such as premature rupture of the membranes, preterm delivery and low birth weight of neonates have been reported to be more common in women with GBS colonization (Bobitt *et al*., 1985). The GBS is an important cause of sepsis, meningitis and pneumonia in the neonatal period (Bobitt *et al*., 1985; Yancey *et al*., 1992; Heath and Anne, 2007). Pregnant women who are GBS carriers are also at risk for sever infections (Bergeron *et al*., 2000; Bobitt *et al*., 1985) such as maternal pelvic infection, puerperal infection and urinary tract infection (Bobitt *et al*., 1985; Matorras *et al*., 1990; Pass *et al*., 1982). Although, some studies have evaluated the establishment of an antibiotic chemoprophylaxis program to prevent the maternal or neonatal GBS infections by intrapartum treatment (Schrag *et al*., 2002; Katz *et al*., 1994; Boyer *et al*., 1983; Van Dyke *et al*., 2009) side effects of antibiotics, increased resistance of bacterial strains and the effect of antibiotics on breast feeding are cause for search of new prevention methods. Some microorganisms are naturally colonized in the urogenital tract and among them; lactobacillus is predominant organism of normal vaginal flora. Lactobacilli play a major role in preventing the colonization of pathogenic organisms in the urogenital tract (Zarate and Nader-Macias, 2006).

In this study, prevalence of recto-vaginal colonization of group B streptococci in pregnant women in a hospital in Northeastern province of Iran was studied and direct Gram stain of the samples were observed to evaluate the existence of normal flora lactobacilli.

MATERIALS AND METHODS

Rectum and vaginal samples were obtained from 201 pregnant women referred to Emam-Reza Hospital in Mashhad (Khorasan-Razavi Province- Iran) from July 2005 to January 2007. Sampling was carried by sterile swab from lower vaginal and rectum tract in third trimester of pregnancy because identifying of intrapartum carriers is important and group B streptococci can spread maternal

or neonatal infection after delivery. Considering the detection more group B streptococcal carriers and report more consistent patterns of colonization, sampling was carried out from rectum and vagina. Two samples were taken from every region, one for direct Gram stain and the other for inoculation to Todd-Hewitt broth (Difco) containing 50 μg mL^{-1} of nalidixic acid and 5-7% defibrinated sheep blood. After 24-48 h the resulted broth culture were subcultured onto 5-7% sheep blood agar. All tubes and plates were incubated in the candle jar at 37°C for 24-48 h. Suspicious streptococci colonies were presumptively identified by colony morphology, hemolysis pattern, microscopic features using Gram staining and biochemical tests such as catalase$^-$, bacitracin (0.04 U) resistance, CAMP, sodium hippurate hydrolysis$^+$ (Matorras *et al*., 1988). Bacteria with positive CAMP test showed arrow-shaped zone of enhanced hemolysis against *Staphylococcus aureus* on sheep blood agar under aerobic condition. Group B streptococcus has the ability to hydrolyze sodium hippurate and produce benzoic acid and glycine by enzymatic reaction. Two colonies of suspicious streptococci were inoculated to 5 mL heart infusion broth (Difco) containing 1% sodium hipporate into screw cap tube. The tube was incubated at 35°C for 48 h and then 0.2 mL of 12% ferric chloride reagent was added to the 0.8 mL of culture supernatant. Formation of a persistent brown precipitate indicated positive test (Facklam *et al*., 1974).

Identifications of group B streptococci were confirmed by a slide agglutination technique (Strep slide). The slides of direct Gram stain were carefully examined for Gram positive streptococci and long Gram positive bacilli with chain arrangement suspected to lactobacilli.

If the result of vaginal or rectum culture was positive, degree of colonization was reported according to colony count on blood agar (Anthony *et al*., 1981; Hoogkamp- Korstanje *et al*., 1982). The degrees of growth in the plates were recorded as follows:

Degree of colonization*	Colony count
1^+	<10
2^+	10-30
3^+	30-50
4^+	>50

*1^+ and 2^+ were taken light infection and 3^+ and 4^+ were taken high infection

RESULTS

As shown in Table 1, 25 (12.44%) of 201 recto-vaginal samples were positive which 24 (11.94%) were positive for rectal swabs. Ten (41.67%) of 24 rectal samples had light infection and 14 (58.33%) indicated high infection. Twenty two (10.95%) of tested women were positive for vaginal samples, of which 9 (40.91%) had light infections and 13 (59.09%) were positive with high infections (Table 2).

Table 1: Recto-vaginal colonization of Group B streptococcus in 201 tested pregnant women

	GBS carriers		Non-carriers	
Samples	%	n	%	n
Vaginal carriers	10.95	22	89.05	179
Rectal carriers	11.94	24	88.06	177
Vaginal and rectal carriers	12.44	25	87.56	176

Table 2: Degree of colonization in vaginal and rectal samples of GBS carriers

	Light		Heavy	
Carriers	%	n	%	n
Rectal	41.67	10	58.33	14
Vaginal	40.91	9	59.09	13

Table 3: GBS carriers based on age

Age (years old)	No. carriers/ No. pregnant in the group (%)
16-20	9/56 (16.07)
21-25	9/57 (15.79)
26-30	4/43 (9.30)
31-35	3/27 (11.11)
36-40	0

Table 4: Maternal colonization by GBS and diabetes

	GBS carriers		Non-carriers	
Groups	%	n	%	n
Diabetes (n = 7)	28.57	2	71.43	5
No diabetes (n = 194)	11.86	23	88.14	171

In the majority of cases, prevailing bacteria observed in the slides of direct Gram stain were Gram positive bacilli suspected to be lactobacilli. In the cases of high infection with GBS, lactobacilli were observed as trivial in direct Gram stain.

The mean age for GBS carriers and no carriers were 23 and 25.5 years old, respectively. Results shown in Table 3 indicate that the rate of transmission among women of 16-20 (9 of 56 pregnant women in this group) and 21-25 (9 of 57) years-old groups were more than other age groups. There were no carriers among the 18 women older than 35. There were 7 pregnant women with diabetes among the sampled group, 2 (28.57%) of which were GBS carriers, compared to 23 (11.86%) GBS carriers among the non-diabetic pregnant women (Table 4). Both vaginal and rectal samples of diabetic women were positive.

DISCUSSION

Group B streptococci are found in different sites of healthy adults, but investigators have paid close attention to its vaginal colonization. The rate of GBS vaginal transmission is very different (Patterson, 1991). Since, GBS is one of the major causes of neonates death and neonates are infected in the uterine or when passing

through delivery canal, studying prevalence of maternal GBS colonization, finding risk factors and preventing maternal and neonatal infections are significant. The GBS transmission among rectal and vaginal specimens of pregnant women using culture and latex agglutination procedure was reported 20.6% in the United States (Park *et al*., 2001). Carriage of group B streptococcus in pregnant women from UK has reported 21.3% (Jones *et al*., 2006). The GBS colonization was confirmed in 23% of pregnant women in Tanzania (Joachim *et al*., 2009). In this study, the rate of GBS recto-vaginal colonization was 12.44% among studied pregnant women and the prevalence of GBS colonization was found less than some other studies. Rate of GBS isolation is different and depends on selected culture media, the number of sampled areas and probably socioeconomic condition and geographic location. In this study, a bigger percentage of pregnant women (11.94 against 10.95%) carried this organism in the rectum. Anthony *et al*. (1981) showed 17 and 20% of pregnant women were GBS carriers in genital and rectal region, whereas Hoogkamp-Korstanje *et al*. (1982) attained 7.9 and 10.6%, respectively. In some studies GBS has been reported more from rectum than vaginal tract (Badri *et al*., 1977; Hoogkamp-Korstanje *et al*., 1982). It is likely that gastrointestinal tract plays a major role in spreading of GBS. In other words, vaginal colonization is a sign of infection from this region (Badri *et al*., 1977; Anthony *et al*., 1981). A higher proportion of pregnant women in the present study had heavy colonization and it is reported that the likelihood of neonatal colonization at birth is higher if the mother is heavily colonized (Patrick *et al*., 2002).

In this study, GBS was isolated more frequently from women younger than 25 years compared with women aged more than 26 years, which is in contrast with a study done in Tanzania by Joachim *et al*. (2009) in which GBS was isolated more frequently from women of age group 30-34 compared with women aged <20 years.

Diabetic women are more susceptible to infection. Some researchers have shown a higher prevalence of GBS colonization in diabetic pregnant women (Faro, 1981; Embil *et al*., 1978; Matorras *et al*., 1988). In this study the percentage of GBS carriers was higher among diabetic pregnant women than non diabetics (28.57% against 11.86%), however more studies are needed to establish this relationship.

CONCLUSION

Since, some complications seem to be more common in women with GBS colonization and their neonates, screening for GBS among pregnant women is crucial. In this study, Gram positive bacilli suspected to lactobacilli in direct Gram stain of GBS carriers were observed less than non-carriers. Considering the significant role of lactobacilli of vaginal normal flora in prevention of pathogenic colonization via acidic pH establishment and other mechanisms, it is suggested to promote the use of lactobacilli probiotics to replicate a controlled vaginal colonization in pregnant women to prevent GBS infections in mothers and their neonates, as an alternative to prescribing antibiotics with their inherent disadvantages.

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